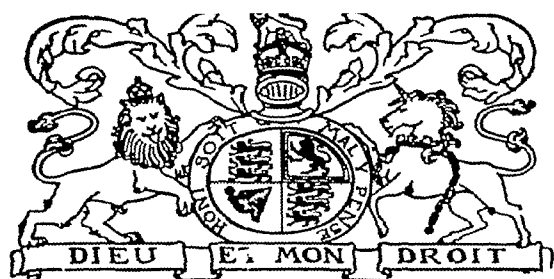




THE INDIAN JOURNAL OF MEDICAL  
RESEARCH





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# THE INDIAN JOURNAL OF MEDICAL RESEARCH

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# A CONTRIBUTION TO THE PATHOLOGY OF CUTANEOUS RAT LEPROSY

BY

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[Received for publication, January 16, 1928]

THE occurrence of a disease of rats due to an organism morphologically similar to Hansen's *Mycobacterium lepræ* was first described by Stefansky in 1903. Since this time the disease rat leprosy has been noted in different parts of the world by Rabinowitsch (1903), Brinckerhoff (1910), Ehlers, Bourret and With (1912), Marchoux and Sorel (1912), Paz de Azevedo (1913), Philibert (1914), Leger (1919), Uchida (1922), and others. The pathology of the disease, as it occurs under natural conditions, has been dealt with by Dean (1905), Marchoux and Sorel (1912), and Currie and Hollmann (1912).

While it is recognised that human leprosy and rat leprosy may be quite different clinical entities, there are undoubtedly points of resemblance between the two diseases: for example, similarity in morphology, staining reactions and resistance to artificial cultivation of the organisms concerned, the relatively long incubation period and the almost strict specificity of the diseases, and, finally, the chronic and non-fatal nature of the infections both in man and in the rat. With such analogies before us, it was deemed that a study of the skin lesions in rats might throw some light on the pathology of naturally-acquired human leprosy.

## METHOD OF EXPERIMENT

Suspensions of rat leprosy material rich in the specific organism were inoculated subcutaneously in the groin region of healthy adult white rats. It has been shown that the percentage of positive results by this method is a relatively high one, varying between 90 and 100 per cent (Muir and Henderson, 1928). In view of our present inability to grow the bacillus of Stefansky on artificial culture media, accurate estimation of the dosage is impossible but the following method was adopted as a working basis —

A piece of highly lepromatous rat tissue was excised, cut into small fragments, ground up with fine sand and diluted with saline. The gross suspension

thus made was then centrifuged at moderate speed for five minutes, the supernatant fluid pipetted off and examined for the presence of bacilli, and, finally, diluted with normal saline until it equalled in density a given standard opacity tube. Of this 'standardised' suspension,  $\frac{1}{2}$  c.c. was inoculated subcutaneously in the groin region of healthy adult white rats. The animals having been killed at different intervals following the dates of injection, portions of skin from the inoculated areas together with the related lymphatic glands were then excised, fixed, embedded in paraffin, cut and stained both by hæmatoxylin and eosin and by Ziehl-Neelsen's method.

A subsidiary experiment to the main investigation was also carried out —

Rats previously inoculated with rat leprosy were injected at intervals with 0.5 c.c. of a one per cent solution of trypan blue. Both the subcutaneous and the intraperitoneal routes of administering the dye were employed. Portions of infected skin together with the related lymphatic glands were excised in the usual manner and the resulting sections stained (a) by hæmatoxylin and eosin, (b) by carbol fuchsin only.

#### HISTO-PATHOLOGICAL APPEARANCES

(a) Examination of the skin of *normal healthy rats* reveals one or two features of interest. The epithelium of the rat's skin is relatively thin and appears to be composed of two layers only, (1) a deep layer corresponding to the basal-celled (or Malpighian) layer of the human epidermis, and (2) a more superficial horny-like layer resembling the stratum corneum of man. Sections stained by Ziehl-Neelsen's method bring out particularly well the prevalence of the so-called 'mast' cells. The origin of these elements is obscure but they are probably a variety of connective tissue cell and quite distinct from the so-called 'mast' cells of the blood. In healthy human skin they average about one in every other medium power field of the microscope, while in many fields none may be detected (Macleod, 1903). In the normal rat skin they appear to be relatively more numerous and they are scattered through the corium in considerable numbers. Irregular both in size and in shape they present in their typical form a bright blue stained nucleus surrounded by cytoplasm, packed with dark red fuchsin-stained granules. In other cases, however, the granules are so abundant that they completely obscure the nucleus. In hæmatoxylin-stained sections the granules appear dark-blue in colour. Masses of extra-cellular granules, obviously the remains of broken down 'mast' cells, are also scattered through the tissues.

(b) Sections of skin excised at varying intervals from *twenty-four hours to eight days after subcutaneous inoculation*, reveal the following characters in varying degree —

There is infiltration of cells in the deeper layers of the corium and in the superficial zone of the subcutaneous tissue. The cellular accumulation, disposed more or less parallel to the skin surface, is made up chiefly of small lymphocytes, occasional 'mast' cells and large rather plump cells with an indefinite outline, and a round or oval and usually eccentric nucleus. Bacilli are present in small numbers at this early stage. They are practically all intra-cellular and are situated

within the 'plump' cells just mentioned. The organisms vary to a slight degree in their acid-fast properties and certain of them tend to lose their fuchsin stain after a time. The granules of the 'mast' cells, on the other hand, appear to retain their acid-fast character more or less indefinitely and care is necessary to differentiate extra cellular 'mast' granules from granular forms of the bacilli.

(c) From the second to the eleventh weeks following inoculation there is a progressive increase in numbers both of bacilli and of the cellular elements. The composition of the latter remains as before but owing to their greater accumulation the plump outline of the 'lepra cell' (as we may call the type of cell which contains the bacilli) is modified, and may at times assume a spindle shape not unlike the appearance presented by fibroblasts. In parts of the section in which the infection is most intense, and in which also the accumulation of 'lepra cells' is at a maximum, the cell outlines are quite obliterated and the cells appear to be piled together. In such heavily infected zones one frequently sees amorphous-like areas surrounded by a ring of nuclei, but in none of our sections have we been able to detect true giant cell formation. The appearance of the nucleus of the 'lepra cell' varies also: in lightly parasitised cells a homogeneous chromatin network can be made out while in cells which are heavily infected the chromatin appears to be fragmented, the nucleus is undergoing karyorrhexis. We have repeatedly confirmed the observation recorded by nearly all students of the pathology both of human and of rat leprosy, viz., that the number of bacilli that may be present in a 'lepra cell' without the latter undergoing disintegration is at times enormous and great felted masses of organisms may be found piled up inside a cell.

(d) Until about the *third month* following inoculation there is little tendency for the infection to spread superficially towards the upper part of the corium and epithelium. From this time onwards, however, there can be detected groups of 'lepra cells' and lymphocytes making their way up towards the hair follicles, sweat and sebaceous glands. The underlying muscles also become involved and masses of bacilli both intra- and extra-cellular can be seen actually in the muscle substance. It is not suggested that the true muscle elements take any active part in combating the invasion of the bacilli. It is more probable that there has been a proliferation on the part of the cells in the connective tissue sheaths of the muscle and that this proliferation has led to fragmentation and ultimately to atrophy of the muscle fibres. In the centres of the more densely cellular areas foci of necrosis begin to make their appearance: such areas have an amorphous structure and contain fragments of nuclei while the bacilli are scattered about irregularly and appear to be losing their acid-fast character.

(e) After the *twentieth week* (but varying in different cases), the whole of the corium and the greater part of the subcutaneous tissue are converted into a lepromatous mass containing vast numbers of acid-fast bacilli. Although the process has now extended right up to the epithelium, few or no bacilli can be detected in the latter. Necrotic foci are still more numerous but there is no tendency to liquefaction in any of the foci. The final stage is one of breaking down of the superficial epithelium, secondary infection of the underlying leproma and ulcer formation.

The *lymphatic glands* related to the site of inoculation show a very similar picture 'lepra cells' and bacilli varying in numbers according to the intensity of the infection can be seen infiltrating the true lymphoid elements. Again there is the same tendency to central necrosis in the lepromatous masses, a feature already noted in the cutaneous lesions. There is little evidence of reaction in the neighbourhood of the leproma, and one finds an abrupt transition from lepromatous tissue to normal lymphoid tissue.

Sections of skin and of related lymphatic glands from infected rats previously stained *intra vitam* with trypan blue revealed that in the majority of instances the vitally stained cells are responsible for phagocytosing the lepra bacilli. This was most clearly shown in sections of glands which had been stained by carbol-fuchsin only: the normal lymphoid elements were unstained and did not contain bacilli, while lightly parasitised lepra cells showed collections of dye granules together with the specific red-stained organisms. The only areas in which the dye and the organisms were not found in association were dense lepromatous nodules packed with bacilli and cells and into which the dye was apparently unable to penetrate. It is, therefore, evident that the specific cell of leprosy, the so-called 'lepra cell', is capable of being stained *intra-vitally*. This finding confirms the work of Oliver (1926), who demonstrated that the 'lepra cell' is simply a modified tissue histiocyte.

#### DISCUSSION

The lesion induced by subcutaneous inoculation of rat leprosy material appears to spread for some distance radially from the point of inoculation prior to invading either the overlying or the underlying tissues, in other words it spreads along the lymphatic planes between the corium and the subcutaneous tissue. We, therefore, find in our experimental animals that loss of hair is not a striking feature early in the course of the disease: this is in marked contrast to natural infections in which alopecia is pronounced. With the exception of the lymphocytic proliferation there is practically no reaction in the neighbourhood of the lesions: once this lymphocytic barrier is broken down the process extends both superficially and deeply. Associated with the absence of reaction there is a corresponding absence of giant cells. Both these processes on the other hand occur in certain types of human leprosy and our clinical and histological studies of this disease have brought us to the conclusion that they are present in cases in which there is a maximum effort on the part of the tissues of the skin (and nerves) to restrict the spread of the disease.

The different microscopic appearances in rat leprosy suggest that the disease though chronic is essentially progressive and that there is no attempt at self healing, such as one associates with human leprosy. The necrosis which develops in the centre of the lepromata is brought about entirely by mechanical means—the extensive accumulation of bacilli-laden cells.

Both human and rat 'lepra cells' show a similarity in morphology and in their relationship to the micro-organisms causing the two diseases. It is, therefore, possible that the human 'lepra cell' also is derived from the macrophage (or histiocyte) system.

## SUMMARY

The histological appearances of experimentally induced cutaneous rat leprosy lesions of different ages have been described and the points of contrast and of similarity between this infection and the naturally acquired human and rat infections have been touched on

Thanks are due to Dr Muir in charge of Leprosy Research at the School of Tropical Medicine and Hygiene, Calcutta, for permission to publish these notes and to Dr S Sen Gupta and Mr Sukhamoy Ghosh, assistants in the Leprosy Research Laboratory

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### EXPLANATION OF PLATE I

- Fig 1 Section of normal rat skin  $\times 200$   
    (a) 'Mast' cell  
    (b) Extra-cellular granules
- „ 2 Section of rat subcutaneous tissue 24 hours after inoculation  $\times 900$   
    (a) Group of bacilli inside cell  
    (b) Connective tissue macrophage (or histiocyte) cell  
    (c) Lymphocytes
- „ 3 Section of rat skin 8 days after inoculation  $\times 80$   
    (a) Band-like cellular proliferation in deeper part of corium and  
        upper part of subcutaneous tissue  
    (b) Hair follicle



Fig 1

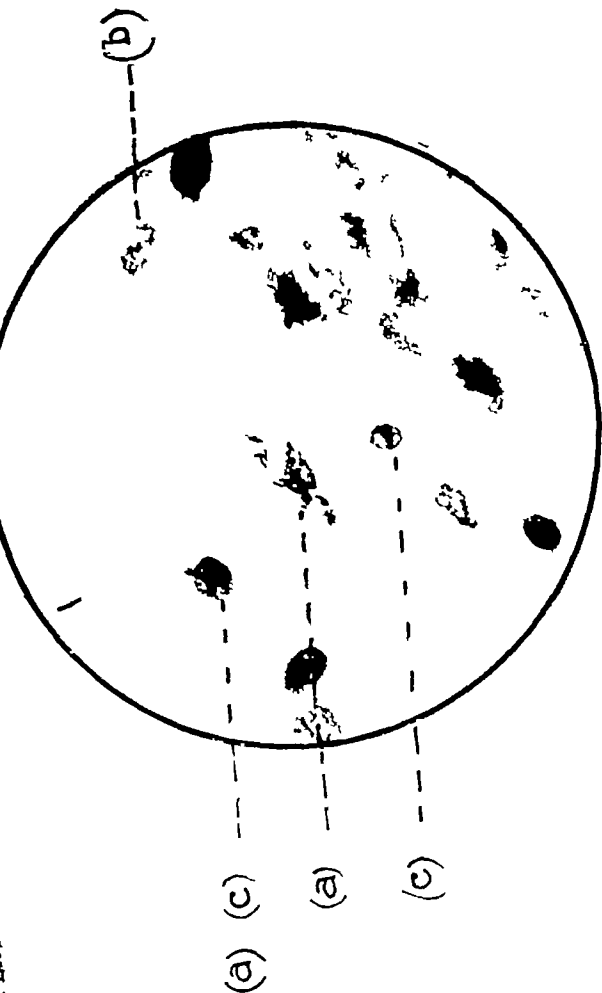
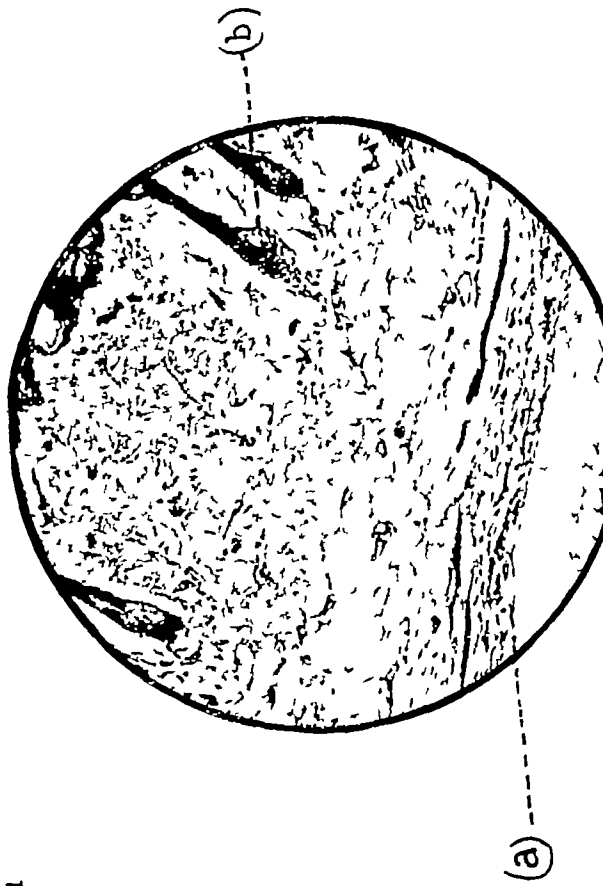


Fig 2









Fig 5

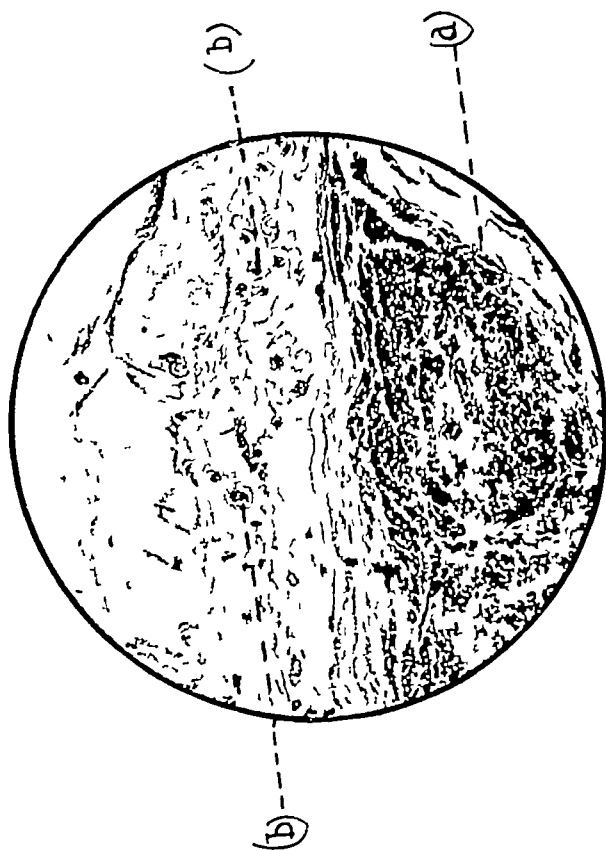
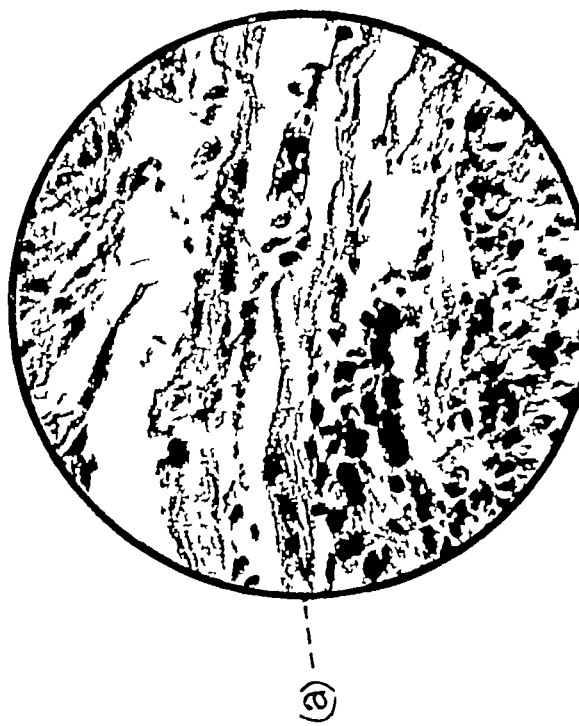


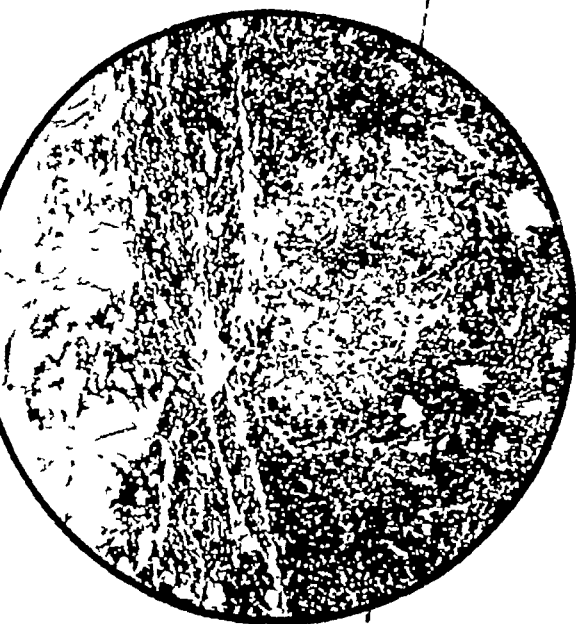
Fig 4

#### EXPLANATION OF PLATE II

- Fig 4 Section of rat skin 7 weeks after inoculation  $\times 40$   
    (a) Dark masses representing accumulations of bacilli  
    (b) Hair follicles in section
- „ 5 Section of rat skin 11 weeks after inoculation  $\times 40$   
    (a) Dark masses representing accumulations of bacilli Note  
        tendency to superficial and deep infiltration
- „ 6 Section of rat subcutaneous tissue 11 weeks after inoculation  $\times 400$   
    Showing cellular infiltration and destruction of voluntary muscle  
    fibres  
    (a) Muscle fibres

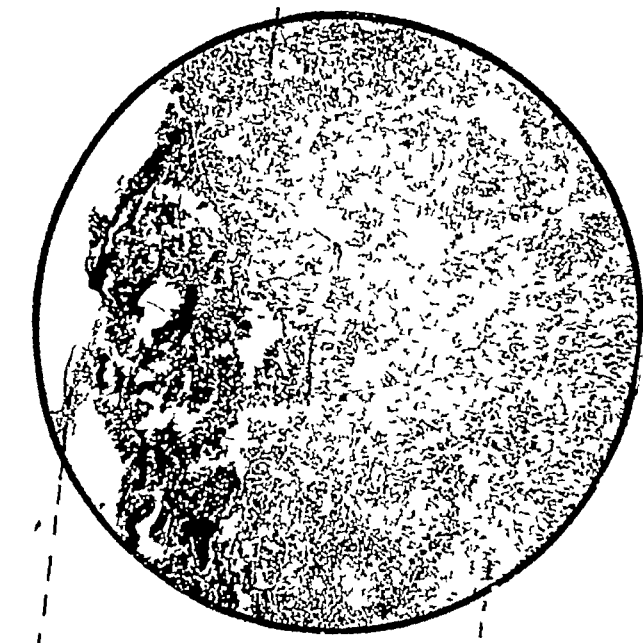
### EXPLANATION OF PLATE III

- Fig 7 Section of rat skin 25 weeks after inoculation  $\times 40$   
    (a) Dark masses representing accumulations of bacilli  
    (b) Area of commencing necrosis
- „ 8 Section of rat skin showing ulceration and abscess formation  $\times 40$   
    (a) Fragments of epithelium  
    (b) Abscess in skin and subcutaneous tissue  
    (c) Remains of lepromatous infiltration
- „ 9 Section of lymphatic gland of rat  $\times 80$   
    (a) Normal lymphoid tissue  
    (b) Lepromatous infiltration



(b)

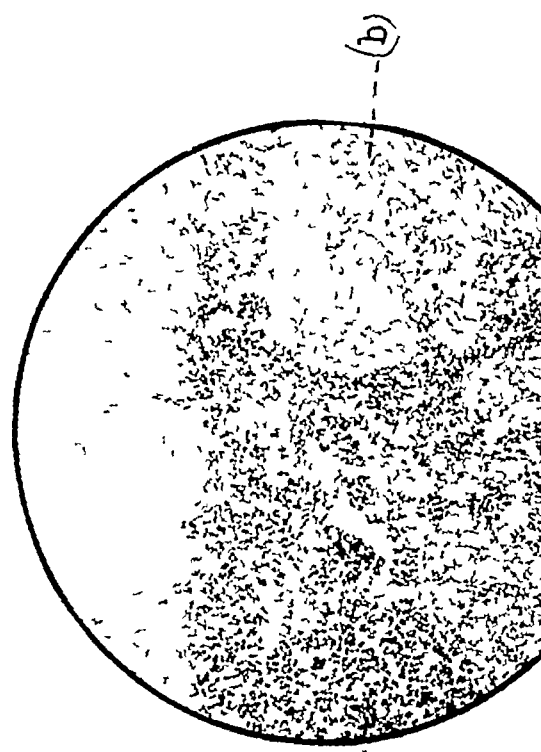
Fig 7



(a)

(c)

Fig 8



(a)

(b)



# THE PRESENCE AND SIGNIFICANCE OF LARGE MULTINUCLEATED CELLS IN LEPROSY

BY

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[Received for publication, February 21, 1928]

THE occurrence of large multinucleated cells in leprous lesions has been noted by various observers among others, Hansen and Looft (1895), Unna (1896), Macleod (1903), Stelwagon (1919), and more recently Wade and Rodriguez (1927), have described the incidence of this phenomenon. The present note is an attempt to correlate this particular histological finding with the clinical signs and to estimate, if possible, its significance in the course of the disease.

## MULTINUCLEATED CELLS IN GENERAL

For descriptive purposes multinucleated cells may be divided into three groups (a) those associated with the infective granulomata of which the classical prototype is the 'giant cell' of tuberculosis, (b) the so-called 'foreign body' giant cells, a group closely allied to if not identical with the foregoing, (c) osteoclasts of bone. Into the controversial topic of the precise mode of formation of multinucleated cells we do not propose to enter. From his tissue culture work on tubercle formation Maximow (1924, 1925), appears to have demonstrated conclusively that in this disease, at least they are derived from the epithelioid cells by a fusion of the latter with disappearance of cell limits and amitotic or sometimes mitotic division of the nuclei.

## MULTINUCLEATED CELL-FORMATION IN LEPROSY

In the course of investigations involving the examination of a large number of sections from different types of lesion we have detected the occurrence of multinucleated cells in two situations only, (1) in the erythematous type of skin lesion, (2) in the thickened and infiltrated branches of a cutaneous nerve or nerves found in association with such a lesion. On macroscopic examination this type of skin lesion is found to be divisible into two groups, (a) in which there is a slightly raised and uniformly erythematous lesion of varying but usually relatively



small dimensions, (b) the 'zone' type of lesion, larger on the whole, and in which there is a flat and partly depigmented area of skin surrounded either wholly or in part by a raised erythematous border. Microscopic examinations incline us to the view that the latter represents *histologically* a more advanced phase of the former, i.e., that the central flattened area is essentially of the nature of a scar.

Clinical examination for the presence of the cardinal signs of leprosy, viz., superficial anæsthesia and the presence of *mycobacterium lepræ*, results in findings which are rather variable. In the raised and uniformly erythematous plaque there is commonly either a slight blunting of the sense of superficial touch or the actual presence of paræsthesia. Clip smears from such a lesion are frequently negative and, even when positive, the number of organisms is usually small—too small to make the procedure of much diagnostic value. In the 'zone' type of lesion absolute loss of superficial touch sensibility is the rule in the centre with blunting of sensation, or the presence of paræsthesia in the raised erythematous border. Adopting the classification proposed by Muir (1927), we may describe such lesions as being of the 'A1—B1' type. Many sections from other cutaneous and nervous manifestations of leprosy have been examined but so far we have not been able to detect large multinucleated cells in any of them. The sections include flat and uniformly depigmented patches both recent and chronic, true nodules both in the quiescent and in the reacting stages, sclerosed nerves, a small number of lymphatic glands and a single example of leprotic testis removed at operation. Investigation of the skin of rats experimentally infected with rat leprosy has also failed to elucidate the presence of these cells (Henderson, 1928).

#### MICROSCOPIC APPEARANCES

The most striking feature on microscopic examination is the definite evidence of resistance on the part of the tissues to the spread of the disease. Lepra cells are present in considerable numbers but instead of swamping the normal tissues of the skin as happens in young nodule formation, they are broken up into smaller foci isolated from each other by circumscribing collections of fibroblasts, young connective tissue and lymphocytes, while at or near the centres of such foci one or more large multinucleated cells are commonly found. A word of explanation is necessary at this point regarding the exact meaning we intend to convey when the expression 'lepra cell' is used. Hitherto this phrase has been restricted to cells of debatable origin occurring in leprous lesions *and containing acid-fast mycobacteria lepræ in varying numbers*. As explained below, the lesions under consideration contain organisms in relatively scanty numbers and we apply the term 'lepra cell' in a strictly *histological* sense irrespective of the bacterial content of the lesions, i.e., exactly in the same manner as the phrase 'epithelioid cell' is used in describing tuberculous lesions. With the notable exception of central caseation and necrosis, the appearances presented closely resemble tubercle formation—the centrally placed large multinucleated cell corresponding to the tuberculous giant cell, the lepra cells very similar in morphology and possibly also in origin to the epithelioid cells, and the peripheral zone of lymphocytes, fibroblasts and young fibrous tissue common to the two infections.

PLATE IV



Fig 1 Erythematous lesions of the 'Zone' type



Turning now to the morphology of the large multinucleated cell itself this structure varies considerably in size, there is no clear cut margin but the borders of the cell are prolonged into irregular strands which fuse imperceptibly with the reticulum between the surrounding lepra cells. The protoplasm of the cell is finely granular, occasionally vacuolated and faintly eosinophil. The number and the position of the nuclei are very variable in those cases in which they form a ring round the periphery of the cell, the latter presents an appearance indistinguishable from that described as typical of the tuberculous giant cell. The nuclear elements stain blue with hæmatoxylin, show a vesicular structure and a well marked nucleolus is a common finding. In these respects they closely resemble the nuclei of the surrounding lepra cells. Care is necessary to avoid mistaking sections of sweat glands, capillaries and the apices of hair follicles for large multinucleated cells.

The presence of organisms in these lesions is extremely capricious in our experience they are at most few in number and scattered about both in and between the lepra cells in the vicinity in many sections we have failed entirely to discover them and we have not yet seen them actually within the substance of the large multinucleated cells.

#### DISCUSSION

Histological studies have revealed the fact that large multinucleated cells are more common in leprosy than had hitherto been supposed. All our material for this investigation has been obtained from biopsies on a large series of outpatients autopsy specimens on which many previous workers have been compelled by force of circumstances to rely for their histological studies are useless so far as the type of lesion under consideration is concerned since these lesions are not commonly found in the terminal stages of the disease, and tend to disappear on the incidence of the complication which causes death. The reason why this particular type of cell should be confined to the 'A1—B1' type of lesion is, in our opinion, intimately bound up with the essentially low pathogenicity of *mycobacterium lepræ*. In the early flat depigmented patch where the organisms are very few in number, the reaction of the tissues, even in extensively anæsthetic and depigmented areas, is surprisingly slight. Invader and victim are living in a state of mutual quiescence. In those cases in which the disease advances to the next stage there is a much more determined effort on the part of the organism to gain a definite foothold in the tissues, which effort is resisted actively by the tissues giving rise to the appearances described. The leprotic 'follicle' is a much more vascular structure than the corresponding lesion in tuberculosis for which reason central caseation seldom occurs despite this fact, however, there is interference with the nourishment of the most centrally situated lepra cells. While we cannot bring forward any proof in support of such a hypothesis, we would suggest both from clinical and pathological considerations that the multinucleated cell in leprosy is derived by a fusion of lepra cells and that it represents in the first instance an attempted defence mechanism on the part of such cells against impending destruction. The

phenomenon certainly occurs at the critical point in the course of the disease, midway between the mild chronic and almost stationary condition represented by the flat depigmented patch and the extensive virulent infiltration of the tissues manifested in the advanced 'B2—B3' type of case

The relative paucity of bacilli in such lesions as demonstrated by our present methods of staining is a phenomenon of interest and one for which we are unable at present to offer any reasonable explanation Medlar (1926) in his studies on tubercle found, in addition to demonstrable acid-fast bacteria, 'phantom forms' corresponding to the shape and distribution of the bacilli We have not encountered such forms in our studies of the leprous lesions

### SUMMARY

The occurrence of large multinucleated cells in leprosy is described together with the clinical appearances of the types of lesion in which this phenomenon is seen An attempt is made to explain the possible origin of these cells and to estimate their significance in the course of the disease

My thanks are due to Dr Muir, in charge of Leprosy Research at the School of Tropical Medicine, Calcutta, for permission to publish this note

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PLATE V

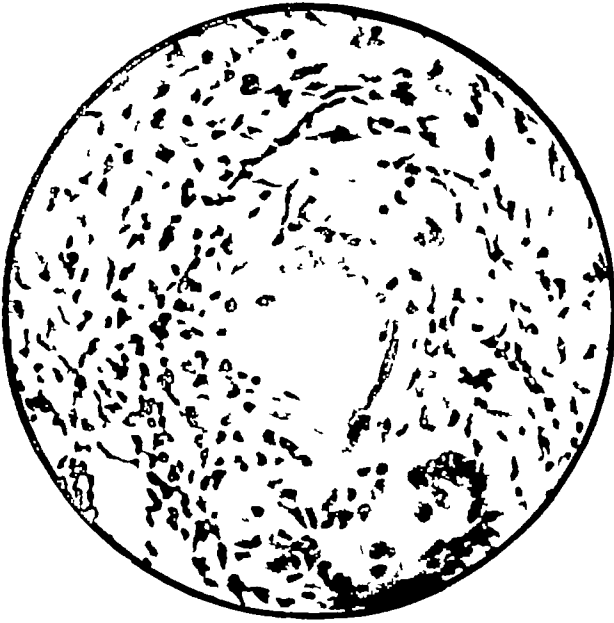


Fig 2

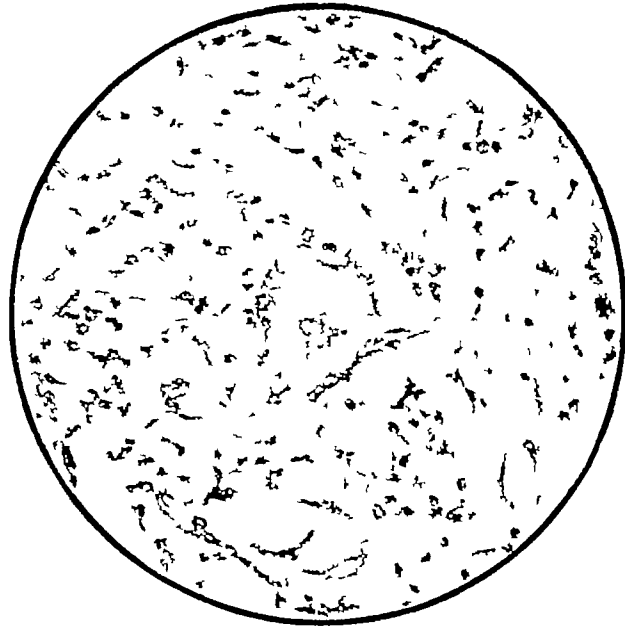


Fig 3

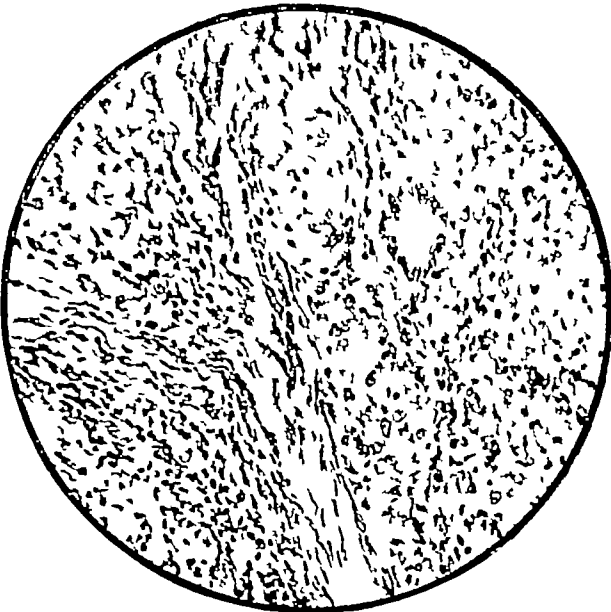


Fig 4

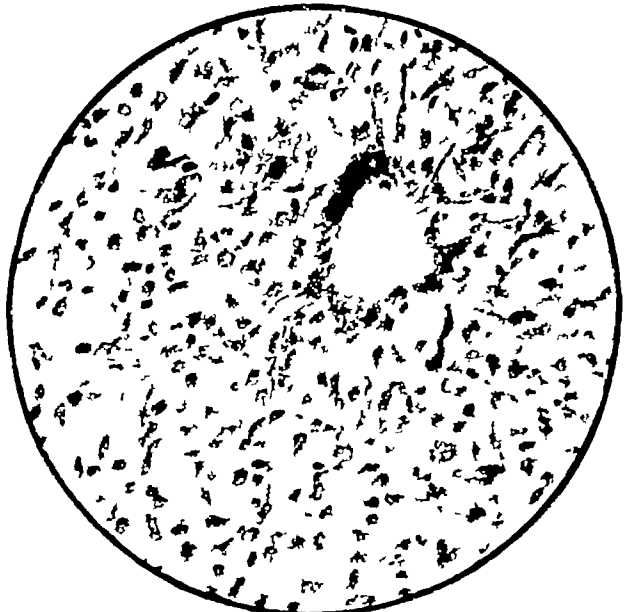


Fig 5

### EXPLANATION OF PLATE V

- Fig 2 Two large multinucleated cells in the centre of a leprotic 'follicle'  
Upper cell shows an exclusively peripheral distribution of nuclei
- „ 3 Large multinucleated cell showing both peripheral and central distribution of nuclei Infiltration of lymphocytes, lepra cells and a few fibroblasts in surrounding area
- „ 4 Large multinucleated cell in an infiltrated and thickened cutaneous nerve considerable fibrous tissue formation in adjacent areas
- „ 5 Tuberculous ovaritis Contrast with Figs 2 and 3





# PHYSICAL FACTORS IN MOSQUITO ECOLOGY

## Part II.

BY

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[Received for publication, January 31, 1928]

### I INTRODUCTION

THE first portion of this paper was published two years ago (Senior-White, 1926) In the present portion it is proposed to give the results of similar investigations extended to other factors as well as those previously measured The work which formed the subject of the first paper was carried out in the submontane districts of Ceylon, that which is now presented has, with a few exceptions specifically mentioned, been carried out on the Indo-Gangetic Plain at Delhi

In Part I the tabulated results of the routine chemical analyses of selected breeding places were not published, only the graphed results being given In the present research too many factors have been measured to graph their various changes with clearness, and as an investigator who has made use of my earlier results has regretted that these were not tabulated (Seymour Sewell, 1927), the results of the present investigation are presented in tabular form

Subsequent to the publication of Part I, there have appeared an important series of papers on similar lines by various Russian authors Sebzentzov (1926), in a preliminary paper, showed that whereas sphagnum-peat waters were unsuitable, sedge-peat waters were favourable to the development of *Anopheles Nikitinski* (1926), in a short paper published at the same time, showed that high pH values favoured the presence of *A maculipennis* (i.e., that it is an alkaliphile), but that hyperoxygenation was apparently inimical As nitrites were not being measured, one wonders if this author fell into a source of error to be discussed in the next section He also gives a list of plankton species indicative of the presence and absence of larvæ Adowa, Nikitinski and Sebzentzov (1927a

1927b) give a series of isolated analyses of selected spots, on which they conclude —

- 1 That the basal distinction between *Sphagnum* and *Carex* marshes, indicated by the third author previously, stands
- 2 That *Clæon* and *Agrion* in particular, with *Daphne*, *Bosmina* and numerous Rotifers are indicative, and *Epitheca* and *Libellula* contra-indicative of the presence of Anopheles

Adowa and Sebenzov (1927) have continued the investigation on the same lines during the following season. They give spacious tables of plankton findings, but their chief conclusion is that it is conductivity which is indicative of the threshold of Anopheles breeding, and that high values are favourable. All their values, however, are low as compared with my Delhi figures, and are nearer my Ceylon 'running water' values.

Owing to their association with numerous systematists, these authors are enabled to present imposing lists and tables of specific determinations of plankton constituents which are quite impossible to a solitary investigator dealing with zoological groups as little studied as are such in Asia. On the other hand, considering that according to the last paper quoted, these investigations are now the result of the team-work of five persons, the chemical work appears to be confined to much fewer factors than might have been studied, and they do not seem to have made a continuous study of even selected spots through a season. Their neglect to study nitrite values has already been mentioned. They have likewise omitted the study of ammonia, which, as I shall show presently, appears to be of the highest importance. Save for their discovery of macroscopic indicator species their plankton work, though intensely interesting, has apparently yielded them as little as my own in direct connection with the presence or absence of larvæ.

## II METHODS OF INVESTIGATION

*Hydrogen-ion Values* were measured as described in my previous paper. *Residual pH* (pH) was obtained by bringing the indicated sample to the boil, and measuring after cooling with standard buffer tubes. As this was done in the laboratory, with a comparator, the values are more accurate than the field measurement of actual pII.

*Conductivity* was measured as described in my previous paper.

*Carbonates* were titrated in the field by the methods described by Birge and Juday (1911). In this connection the reader is referred to a recent paper by the present author (Senior-White, 1928c), regarding the accuracy of the formulæ used by the American workers referred to.

*Phosphates* were measured by the method of Deniges (Atkins, 1923), which affords a rapid means of measuring very small quantities colorimetrically. On the other hand the considerable natural coloration of most of the samples examined rendered the results considerably less accurate than they would be in the sea or in clear stream waters, but the values given in the tables are at least sufficiently accurate for comparative purposes.

*Saline* and *Albuminoid Ammonia* were measured by the standard Public Health methods in general use

*Oxygen* was measured, as before, by the Winkler process. A glance at the tables will show that the amounts found are often ridiculously high, being hundreds per cent over saturation values in foul waters. The point was submitted to Dr W R G Atkins, FRS, who, after consultation with Professor Ramsden of Trinity College, Dublin, came to the conclusion that the presence of nitrite in considerable quantities was responsible for the findings, as the nitrous acid released by the addition of the final strong hydrochloric acid also attacked the potassium iodide, releasing iodine, which was of course measured in the final thiosulphate titration along with that released by the manganic oxide, thus entirely vitiating the results. No use, therefore, has been made of the oxygen figures, which are merely tabulated to show to what absurd results the Winkler process is liable to lead one in the presence of much nitrite, and to warn others against a similar error.

*Plankton*—10 c.c. of the water under examination was centrifuged and a sample of the deposit pipetted off and examined under a 1/6th inch objective. Only the more common constituents of the samples are tabulated, with some idea of the prevalence according to the following symbols—

- +++ Extremely numerous
- ++ Numerous
- + Present in appreciable numbers
- +— Rare
- +— — Very rare

Not being myself a competent algologist no attempt has been made to take the determination further than genera, and even some of these are given with considerable reserve.

*Larval Density*—Up to June this was not measured, the numbers recorded being those actually taken in sampling, but from July onwards ten dips of a large ladle were made in each breeding place, and the results of the count are recorded, unless larvæ were very numerous, when fewer dips were made and the count multiplied to represent ten dips.

Before leaving this point it is my pleasant duty to record my thanks to Dr G A K Marshall, FRS, of the Imperial Bureau of Entomology, for getting numerous determinations of insects, other Arthropods and algæ made for me in London, to Lieut-Colonel H Seymour Sewell, IMS, and his staff on the Zoological Survey of India, for determinations of several specimens of fish, etc., to the Botanical Survey of India, for naming Phanerogamic plants, and to my Colleague, Capt P J Barraud, for checking the determinations of several of the more difficult Culicini. Finally I would express my indebtedness to Sub-Assistant Surgeon Tulsı Ram, in charge of the Public Health Laboratory, Delhi, for carrying out the whole series of ammonia analyses, a time-taking process beyond my time working single-handed. The routine analyses of the water supply of Delhi are done by the laboratory, and I think the results can be accepted without the slightest qualms.

## III THE LOCALITIES STUDIED

The spots chosen for routine investigation of the mosquito fauna and the physical factors measured in relation thereto were selected in the course of operations connected with a malaria survey of Delhi, published elsewhere (Senior-White, 1928a) They must now be briefly described

1 *Roshanara Garden Tank*—An ornamental tank of about four acres area, fed by water from the Western Jumna Canal (Plate VI, fig 1) The banks are grassy, there are patches of *Typha* in various corners, with considerable growth of *Nelumbium* over the surface in the latter half of the year The bottom flora consists of *Potamogeton crispus* and *Vallisneria spiralis* with a considerable amount of *Chara* sp The phanerogams seemed to occur rather in separate patches, whilst the *Chara* died down in the spring and was not thereafter prominent

Table I gives the result of the analyses, together with the various species taken at the same time, and the microfloral record So far as there seem to be any relationships between the algæ and the mosquitoes, which from the table are not very apparent, they seem to be as follows—The *Rhizoclonum*, a filamentous alga, supported of itself a very numerous microflora, the synbion being as follows on a sample taken on February 28th, 1927 *Rhizoclonum incroglyphicum*, on it *Epithemia*, *Gomphonema*, *Cymbella*, *Lyngbya æstuaru*, *Anabæna* (two spp near *sphaeroides* var *tenuis*), *Ædogonium*, *Microchæte* (? *uberimma*), *Tetraspora* sp, *Protophora*, *Nodularia spumigena*, *Closterium* (near) *dianæ* Therefore, whilst it was present, until the end of May, there was ample larval food available *Sphærocystis* rendered the water actually cloudy in the late spring, and its maximum seems to coincide with the spring abundance of *A fuliginosus*

The place of *Sphærocystis* was taken by *Planktonema* (determination doubtful) after the onset of the monsoon, with a complete disappearance of the *Rhizoclonum* until October The short filaments of *Planktonema* did not appear to furnish as good a pabulum as its predecessor, and no algal dominance seems to account for the heavy breeding of *culicifacies* and *fuliginosus* between July 16th and August 8th, nor for the complete cessation of breeding in the following week Turning to the chemical factors, phosphate varies so irregularly that it explains nothing, the big rise in ammonia accounts for the sterility of February 28th, but not for that of August 15th, when a rise to an inhibitory figure only followed a week later

Though this is a typical Indian tank, its analyses furnish very little aid to the factors underlying the seasonal changes in the Anopheline production of such None the less they are given as illustrating, it is believed for the first time, the annual cycle therein

2 *A Water-logged Irrigation Area* (Plate VI, fig 2) This is a state of affairs typical of many localities in North India Irrigation has so raised the water table that the land is sour, under the sun noxious salts effloresce on the surface of the soil, and agriculture is rendered unprofitable The spot in question is the tail end of the Wazirpur distributory of the Western Jumna Canal, and





TABLE 1  
*Irrigation Channels*

[illegible]





though the land around is no longer usable for crops, excess irrigation water runs into these ditches to an extent, and at intervals, depending on the actions of the landowners nearer the offtake. In Table II, the periodical inrushes of canal water are shown by large drops in the conductivity. At other times the channel contains stagnant and slowly evaporating water. No attempt is, of course, made to keep the now useless ditches clean or free from grass, in this case mainly *Zamchiella palustris*.

The results of the analyses are given in Table II. Again we see saline ammonia inhibitory on two occasions, February 28th and June 2nd. There are two exceptional rises in the phosphate value, the first associated with an outburst of a *Spirillum*-like bacteria and with Chlamydomonads, but the second with no noticeable plankton change, whilst a second Chlamydomonad outburst is not associated with any phosphate rise.

From October onwards, when with a single exception on October 31st, associated with rather plentiful *C. tritæmorhynchus* and *C. visknu*, the reaction of the water was approaching neutrality, there being considerable free CO<sub>2</sub>, the algal plankton available for food was exceptionally low and the breeding extremely poor.

An interesting point confirmatory of a recent note on this species (MacGregor, 1927), is the single appearance of the predaceous *Lutzia fuscana* associated with very plentiful *C. tritæmorhynchus*. These larvæ do not, as MacGregor points out, so much hunt as lie in wait for their prey, and hence a certain concentration of the species fed upon is necessary for them to flourish.

Apart from these points, however, there is nothing in the table explanatory or even suggestive of the reasons for the comings and goings of the Anophelines. During the season the place yielded more actual species of mosquitoes than any other breeding place examined.

3 *River Floods*—Such an area was specially examined in connection with the peculiar malaria problem of Delhi, the Bela riverain area of the Jumna. The results are presented in Table III, whilst Plate VI, fig. 3 shows the appearance of the locality at maximum flood. The first week's figures are those of rain pools on the same site before the river rose and flooded the area, which occurred on August 4th, and the examinations were continued until the final figures are those of a foul relict puddle drying under a hot sun.

It will be seen that there is a great difference between the rain and the river water, in regard to phosphate content. The river water is exceptionally low in this respect until it has been on the land nearly two weeks, and breeding commenced at this point. The absence of breeding from the rain pools is probably due to their extremely high temperature. The entrance of *A. culicifacies* coincides with the minimum saline ammonia value.

The microflora of the rain pool was very different to that of the flood water, being mainly Cœlastraceæ, whilst Diatoms appeared with the river water. The very high amount of nitrite present in the rain water and again during September is revealed by the absurdly high 'oxygen' figures of those periods.

Here again we see no connection between the microflora and the amount of breeding, the maximum of the latter coinciding with a very poor microfloral period

4 *A Rain Pool*—This was examined as a typical example of the purely temporary breeding place so common everywhere during the monsoon. The original depression in which that examined was formed was probably the result of taking a small quantity of earth for road repairs or village hut building, the area of the pool at its maximum being about ten feet square. As Table IV shows, it dried between periods of heavy rain for varying lengths of time. The predominant mosquito was as is usual in such cases, *A. rossii* but there was a short period when a very heavy brood of *A. culicifacies* was produced. This coincides with a considerably lower temperature than the previous rain filling had led to, but on this ground there is no reason why the malaria-carrying species should have been absent from the final filling in October, which yielded Culicines only. No chemical or plankton factor serves here again to furnish any clue to the faunal changes.

*A Borrow Pit* (Plate VII, fig 4)—So much has been written of the dangers of borrow pits that any information thereon can hardly fail to be of value. That, studied (Table V), is perhaps not altogether typical in that it collects surface washings from the made land on the Delhi Bela filled with a mixture of night-soil and city refuse, but in India, where any waste land near a town is invariably used as a public latrine, this borrow pit becomes only an exaggerated case. The study is commenced from the filling up of the dry pit with the onset of the monsoon, it having previously been dry since the middle of April, i.e., for three months, but the analyses made during the final part of the drying-off process at the beginning of the year are appended for study.

Only two species, both Anopheline, formed the Culicid fauna. *A. rossii* was present almost throughout, only failing during the first three weeks of September when the microflora, in spite of high phosphate content, which was here a continuous phenomenon, was very scanty. The larval count of November 14th is not given, the water was a mass of eggs and just hatched larvæ.

The factors at work in the entrance of *culicifacies* are as obscure as ever, its exit, though the examination of November 7th resulted in one of the few exceptional records for this species, is obviously controlled by ammonia.

6 *Foul Pool* (Plate VII, fig 5)—It is a truism that of the many bodies of water examined by any worker, few by comparison are found to be breeding larvæ, and, of the remainder, there are none less likely to do so than foul, often green, pools, in spite of the abundant microflora, including *Clathrocystis*, that such usually contain\*. Whilst this is so, the reason has not been studied, and for the present purpose there is nothing more likely to yield results than the comparison of a good breeding place, such as that in Table I, with a not dissimilar sized piece of water that is steadily sterile. Such has been found in the Shahji Tank, outside the South Wall of Delhi City, which, throughout a weekly series of examinations over ten months of the year, has never yielded a single larva of

\* This has recently been confirmed for Malaya by Williamson [*Bull. Ent. Res.*, XVIII, 433]





Oscillatoria	Lyngbya	Melosira	Diatoms	Closterium,	Netrium	Euglenoids	Chlamydomonads	Pleurococaceae	Coleps	Spirulina	Scenedesmus
+++	+	+-	+-	+-		+- +-- +- +- +- +- +	+-      +- +-- +--	+-			
		+	+-								
		+-	+-								
		+	+			++					
			+-			+	+				
			+	+		++	++				
		+		+		++	+				
				+-		+-	+-				
			+-								
			+++								
			+-			+	+++			+	
			+++				+				
							+++				+-



[illegible]





any species In the annual reports of the Medical Officer of Health, Delhi, this tank was for many years put down as a bad source of Anophelines, for which reason it was originally included in the spots for weekly examination That it has yielded the results now made use of was entirely unforeseen The reason for the change in status was not appreciated until it was realized that whereas this tank was formerly fed by the Western Jumna Canal, it is now dependant entirely on rainfall, and thus becomes fouler and fouler It is now of little use to the people save as a buffalo wallow, for which it is largely used It never dries out completely, but its chemical history is commenced from its refilling by the monsoon However, the analyses made earlier in the year up to the time that the part examined, shown dry in the foreground of the photo, dried up, are included to show the state of relict puddles of this nature The water is at all times brown and filthy, though at times dull green with Euglenoids, mainly *Euglena oviformis*, which on March 10th, counted up to 100 per c mm

These analyses at once show why the pool is Anopheline sterile, the ammonia is at all times present in absolutely inhibitory amounts Phosphates are always high, usually in enormous excess It is doubtful if free oxygen is ever present in such filthy water, the small amounts found being probably in all cases only nitrites *Euglena* seems to be present in large numbers only in the absence of free  $\text{CO}_2$ , i.e., at a pH  $> 8.4$

#### IV THE FACTORS CONSIDERED SEPARATELY

In the foregoing sets of analyses of typical breeding places on the Indo-Gangetic Plain eleven physico-chemical factors and the qualitative constitution of the microflora have been examined Of these, as I stated in a preliminary paper (Senior-White, 1928b), the majority have proved disappointing, and it now remains to consider them in detail

*Surface Temperature*—Only Hodgson, who published two papers on the subject, Hodgson and King (1914) and Hodgson (1920), seems to have done any work on this subject until recently, when field observations were supplemented by some experimental work by Rees Wright (1927) Before considering my own results, the work of these authors must be summarized

Hodgson and King (1914), working at Delhi and Madras, came to the conclusion that the internal temperature of the larva was that of the wet bulb of the thermometer, and state that the optimum temperature for *Anopheles* lay between  $68^\circ$  and  $78^\circ\text{F}$  ( $20^\circ$ — $26^\circ\text{C}$ ), whilst temperatures of  $95^\circ$ — $104^\circ\text{F}$  ( $35^\circ$ — $40^\circ\text{C}$ ) were rapidly fatal They also made the important discovery that small hoof-mark pools in grass might be  $5^\circ\text{C}$  cooler than a large pool six inches away, particularly during dry hot weather They concluded that the mosquito shows no sign of being able to regulate its own temperature, and that the great destroyer of larvæ in nature is raised temperature

In his second paper (1920), Hodgson elaborates his work on additional results obtained in the Near East Theatre of War He concludes that the optimum temperature for the growth of *Anopheles* larvæ, for the existence of the adult mosquito, and for the sexual growth of the parasite is in all cases  $60^\circ$ — $70^\circ\text{F}$  ( $16^\circ$ — $21^\circ\text{C}$ ), or about  $5^\circ\text{C}$  lower than his Indian results He also concludes

that eggs are rarely laid in water above  $21^{\circ}\text{C}$ , and that though larvæ manage to exist when the temperature of such water is raised to  $27^{\circ}\text{C}$  or more, the greater number fail to turn into adults, or if they do so, these are weaklings of short life. This inaccessible paper is of great importance and should be studied in detail by all interested in the subject.

Rees Wright experimented with only one Anopheline, *bifurcatus*, regarding which he quotes Buxton (1924) as showing that it is never found at temperatures above  $21^{\circ}\text{C}$  in nature. Wright found that  $32^{\circ}\text{C}$  marks the limiting temperature at which this species was slightly affected in five minutes, the rise of another degree being very prejudicial to its subsequent development. In experiments with raised temperatures continued for an hour, which more nearly approximate to natural conditions,  $32^{\circ}\text{C}$  again marks the limit of resistance.

Turning to my own results the temperatures recorded in Tables I—VI were taken between 8 and 11 a.m., by no means the hottest hours of the day, which are those of early afternoon, as the figures in Table VII show. These were taken on May 4th, when the official meteorological record for Delhi runs —

8 a.m.

Dry	Wet	Max	Min	Humidity	Rain
69.8	65.0	81.0	66.1	75 per cent	0

This maximum is  $31^{\circ}\text{F}$  less than the highest maximum recorded that year,  $112^{\circ}\text{F}$  on May 24th. The heat on the day of examination, however, was for all that nearly insupportable, owing to the high humidity. The findings are given in Table VII, from which we see *stephensi* and *fuliginosus* apparently breeding without restraint up to  $10^{\circ}\text{C}$  higher than Hodgson's Indian upper optimum figure of  $26^{\circ}\text{C}$ , which is more applicable to Indian species than his revised figures based on the Palearctic Anophelines of Palestine.

TABLE VII  
*Anopheline Breeding at High Temperatures*

Place	Average temp a.m.	Temp 3-4 p.m.	$^{\circ}\text{C}$ Diff	Species	28th April		5th May	
					L	P	L	P
Cement water channel	23.5	27.1	3.6	<i>stephensi</i>	11	0	15	0
				<i>culicifacies</i>	17	0	22	0
				<i>fuliginosus</i>			1	0
Irrigation channel (Table II)	25.9	32.0	6.1	<i>stephensi</i>	2	0		
Roshanara Tank (Table I)	25.0	30.3	8.6	<i>fuliginosus</i>	16	1	12	0
Borrow Pits	29.8	34.0	4.2	<i>stephensi</i>	20	1	5	2
				<i>culicifacies</i>			2	0

Now it is noteworthy that Hodgson claims that, though larvæ are not killed, their subsequent development is inhibited. The results quoted show, however, that pupæ were still being taken in small numbers, and continued to be at still higher temperatures later in the month. Reference to the breeding notes of these dates moreover show that of the larvæ brought into the laboratory a high percentage successfully emerged. It seems therefore permissible to query the accuracy of Hodgson's results, and to point out that the whole subject requires re-investigation.

Before leaving this point, however, it may be useful to give the results of all temperature readings taken in connection with larval examinations during the Delhi survey in February–May and July–November inclusive. The values are artificially raised by the small number of examinations made during the cold weather and by the fact that no temperature records were taken in wells, which would undoubtedly have reduced the optimum for *stephensi*. This table gives details for the four common Anophelines of the plains of India, instead of generalizations on 'Anophelines' as given by Hodgson, and the specific splitting of the data into actual species records is interesting. From Table VIII we see the maximum number of findings to be as follows —

Species	Optimum	Percentage of total findings
<i>stephensi</i>	24°C	14
<i>culicifacies</i>	28°C	12
<i>fuliginosus</i>	25°C	15
<i>rossi</i>	32°C	18

That is, there is a very considerably higher optimum for *rossi* than for the three carrier species. These figures are admittedly far too few to generalize upon, in fact it may be not be possible to generalize at all, local races may have different optima, but the closer investigation of the matter is open to every field worker who adds a thermometer to his equipment.

*Hydrogen-ion Concentration*—In the previous part of this paper, I came to the conclusion that individual species had pH optima and tolerance ranges more or less restricted. I am now of the opinion that such do not generally exist, though they can be found in respect of individual localities. In Table IX are given the results of pH observations on twelve species encountered during anti-malaria surveys in a large part of East Central India, ranging from the Vizagapatam Hill Tracts through Western Bengal to the Eastern Central Provinces.

Wide tolerances are seen, with optima nearly always from pH 7.0–7.4, only *rossi* going further into alkalinity, to pH 7.6. On the other hand, these species extend to the acid maximum at pH 5.4. The mean value of all waters examined is 7.0, and of all waters yielding no larvæ 7.2, which I think is conclusive that there is no direct connection in nature between Anopheline distribution and pH.

Similar summations from the Delhi work have been made (Table X), but as no acid waters were anywhere found, and few lower than pH 8.0, the optima for

TABLE VIII  
*Anopheles* Tolerant and Optimum Temperatures  
 Number of times found at °C

Species	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
<i>stephensi</i>			1	1					2	3	3	2	<b>6</b>	2	4	3	4	4	3	1	2	1	1			1	
<i>culicifacies</i>	4	2			1		1		2	3	2	7	9	6	6	5	<b>10</b>	8	6	3	3	5	1	1	1		
<i>fuliginosus</i>	3	1		2	1		1		2	2		3	3	<b>6</b>	3	3	2	4	2	1	2						
<i>rossi</i>							1	1	1	1		2	2	3	3	5	5	9	8	5	<b>15</b>	5	4	3	3	4	1
No larvae	1		3	3	5	4	3	1	5	8	<b>12</b>	7	7	7	6	9	4	10	6	5	9	2	4	2	4	1	1

the four common *Anopheles* of Delhi, three of which also appear in Table IX, are seen to be very much higher than their conspecific relatives in Central India. In every case except that of *rossi*, which agrees in both localities in having the optimum higher than the mean for all waters, it is seen that the specific optimum is only that of the mean of all waters and of all nil waters, and is, therefore, simply the result of normal chance distribution.

In Fig. 1 are given the pH tolerance ranges, and an indication of the optima, where there are sufficient observations, on nine species of *Anopheles* common to two or all the three areas where such observations have been made. The Ceylon results are from Table I of the first part of this paper, those from East

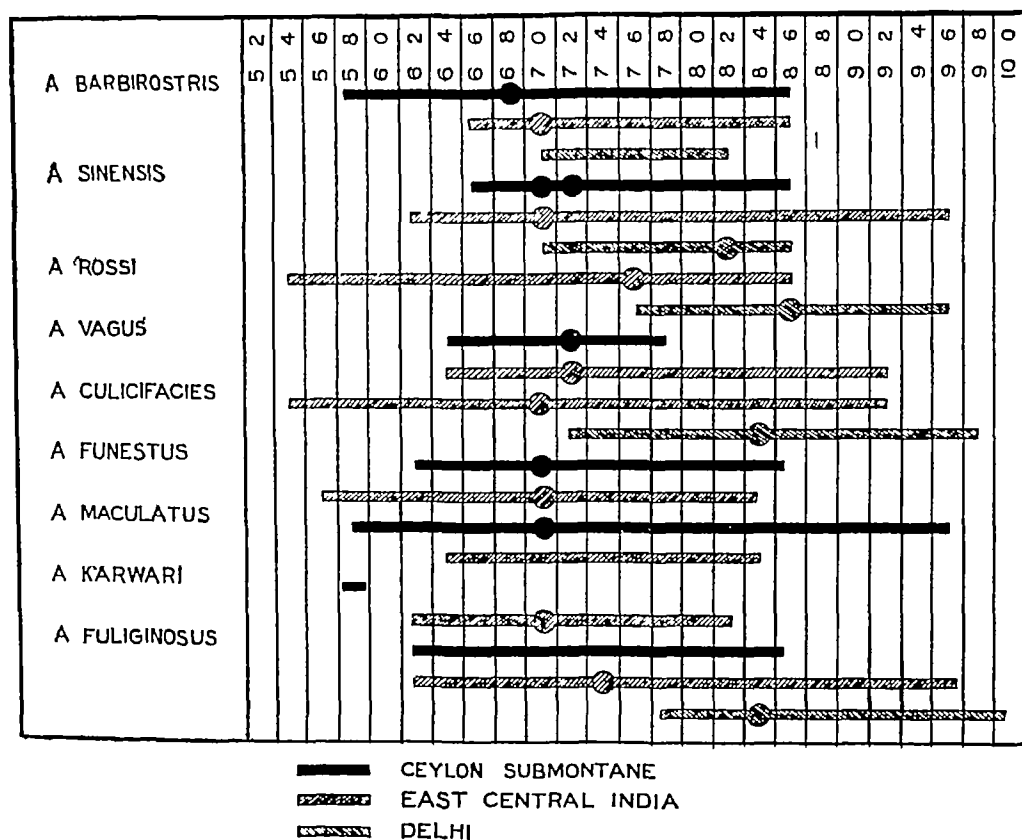


Fig. 1—Anopheline Tolerations and pH Optima

Central India and from Delhi are from Tables IX and X of the present part, with the few observations from the latter place on the two Protoanophelines added. It will be seen that very different conclusions would in most cases have been drawn regarding toleration limits and optima according to the locality in which the work has been done. In no case do the optima coincide where the species is common to all three localities, though in all cases, save the single Ceylon observation on *karwari*, the ranges overlap. The study of pH in connection with Anopheline breeding can therefore be no more than local, without, as was originally hoped, general applicability. Whether there are biological species within the morphological ones, making the latter no more than complices (as has been suggested by the late F. M. Howlett in an unpublished manuscript and

TABLE IX  
E—Central India pH

Species	52	54	56	58	60	62	64	66	68	70	72	74	76	78	80	82	84	86	88	90	92	94	96
<i>barbrosus</i>								2		4	1	3	3	1		3	3		1				
<i>sinensis</i>						1	2	5	4	11	4	9	6			1	4			1			
<i>cultifacies</i>		2	1			3	4	6	5	15	8	8	10	4		4	4		1		2		
<i>funestus</i>			1					3	2	12		6	3	2		3	4						
<i>rosii</i>		1						3	2	4	1	2	9	3		1	6		1				1
<i>lugens</i>							3	4	1	4	5	3	2	3		4	2		1		1		
<i>theobaldi</i>			1					3		1													
<i>maculatus</i>								1									1						
<i>karicani</i>						1		1	1	3	2		1										
<i>maculipalpis</i>								2	1	1		1											
<i>fuliginosus</i>						2	1	1	2	2	1	3	2	1			1						1
<i>pallidus</i>			1				1	4	2	8		3	5	1			1						
No larvæ	2					3	3	4	12	17	18	11	14	9		4	4		1	2			
All findings	4	1				8	13	26	26	57	36	29	42	21		23	25		2	2	2		1

N.B.—The species with no optimum in heavy type have too few records to ascertain this point

as my own work on saline waters suggests), is a matter which can only be tested by the transference of the eggs of various species from one locality where the range is restricted to another where it is wide, or to artificial solutions otherwise suitable for breeding but buffered beyond their normal range. It would be extremely interesting, for instance, to watch results with *Delhi rossii* on the extremely acid seepages of Dongargarh in the Central Provinces, as compared with the local race living therein.

TABLE X  
*Delhi pH*

Species	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6	8.8	9.0	9.2	9.4	9.6	9.8	10.0
<i>stephensi</i>				1		1	3	9	3	6	2		6	4	3	
<i>culicifacies</i>		1		2		4	4	9	6	7	3	1	7	5	3	
<i>fuliginosus</i>					2	2	5	8	1	1		1	3	6	3	4
<i>rossii</i>				2	4	3	4	4	6	5	1	2	4	1		
No larvæ	6	1	1		8	14	11	16	6	5	7	3	3	7		5
All waters	6	2	1	5	14	21	21	39	19	17	10	7	15	16	5	90

Buxton (1927) found that gross differences of pH, however, did have an effect on the oviposition of two species of *Aedes*, which laid most heavily in neutral water, and absolutely avoided those with an acid reaction. This experiment was repeated by me with *A. stephensi* caged with tap and distilled water at varying concentrations less unnatural than those used by Buxton —

Experiment	Water	pH	Eggs laid
I	Tap	7.5	73
	Distilled	5.5	0
II	Tap	8.0	157
	Distilled	5.7	0

The acidity of the distilled water was entirely due to  $\text{CO}_2$ , and, had it been exposed to the air long enough, would have ultimately become neutral. There is here evidence of a distinct preference for an alkaline medium.

*Residual pH* — I am in agreement with MacGregor (1923) that Anophelines are in general alkaliphiles, and my earlier work showed that acidity, to be tolerated, must be due to  $\text{CO}_2$ . The present investigations have not served either to confirm or disprove this, owing to the apparently complete absence of acid natural waters around Delhi. The study is perhaps worth continuing for the sake of confirmation.

*Conductivity* — The present investigation makes it clear that preferences in this respect are a purely local phenomenon. In this is included the existence of halophilic littoral races, the existence of which, stated in Part I, will be further proved by my work at Vizagapatam Harbour when this is published. The conclusions which Adowa and Sebenzov (*vide* Section I) come to on this point in



respect of *A. maculipennis* will probably be no more than a local phenomenon in the Moscow district, and it rests with a worker in England or any part of Europe to follow up this point

*Carbonates*—Nothing has been more disappointing than the results of these laborious titrations. Save for a chemical connection with pH and with conductivity, the subject of a separate paper (Senior-White, 1928c), they have yielded nothing, at least not in connection with mosquito breeding

*Phosphates*—These have likewise furnished no clue to larval abundance either directly or in connection with the microflora on which the larvæ largely feed. The polluted waters around a considerable city do not, of course, form a very suitable subject for the analysis of this point, shown to be so important by Atkins (1923), especially in the sea, and the further study of the anopheline fauna of hill streams in rocky districts, such as Malaya, where I am still of the opinion that a distinct relationship will probably be found to exist, is recommended in this connection

*Oxygen*—As stated earlier, the enormous nitrite content of Delhi waters has completely spoiled this year's investigations. My Ceylon findings in Part I in this respect will be discussed in connection with the next factor

*Saline Ammonia*—From the rough measurements recorded in Part I of this paper, I reached the following conclusion: 'Saline ammonia in amounts of less than one part per million are inhibitory to natural water breeders, especially Anophelines'. The principal result of the present series of investigations is to fully confirm this finding in regard to Anophelines, with the exception of *A. rossii*. I think that the principle thus proved is of sufficient importance to requite one fully for the disappointing results obtained from all the other factors investigated. These ammonia results are tabulated in Table XI and shown graphically, in another form, in Fig. 2

TABLE XI  
*Saline Ammonia Content in Relation to Anopheline Breeding*

NH <sub>3</sub> p p m	Limes found	Nil	S	Larva		R	S	C	F	R
				G	F					
							Per cent	Per cent	Per cent	Per cent
11	1	1								
10										
9	1	1								
8										
7	3	3								
6										
5.5	1	1								
5.0	1	1								

S = *stepensi*, C = *culicifacies*, F = *fuliginosus*, R = *rossii*

TABLE XI—contd

NH <sub>4</sub> p p m	Times found	Nil	S	Larvæ		R	S	C	F	R
				C	F					
							Per cent	Per cent	Per cent	Per cent
45	2	2								
40										
35	4	3		2				05		
30	3	2	2				08			
25	7	3	5	2		280	21	05		366
20	5	4		1				03		
19	2	2								
18	2	1				9				12
17	1	1								
16	1	1								
15	1	1								
14	2		3			110	12			144
13	1	1								
12	5	5								
11	3	3								
10	1	1								
09	2				2	56			07	73
08	6	2	13		11	47	53		41	61
07	6	4		33		26		85		34
06	9	4	25		19	13	103		70	18
05	12	6	44	10	4		181	26	15	
04	8	3	28	26	5		115	67	19	
03	19	4	90	56	31	89	370	145	113	116
02	27	5	28	117	114	50	115	303	421	65
01	35	14	5	92	47	81	21	238	174	106
00	7	1		47	38	4		122	140	05
	178	80	243	366	271	765				

S=*stephensi*, C=*culicifacies*, F=*fuliginosus*, R=*rossi*

One hundred per cent of the *fuliginosus* 98.6 per cent of the *culicifacies*, and 95.8 per cent of the *stephensi* larvæ have been found at values of not more than one part per million. *A. rossii*, on the other hand, has 53.2 per cent of its larvæ beyond that figure.

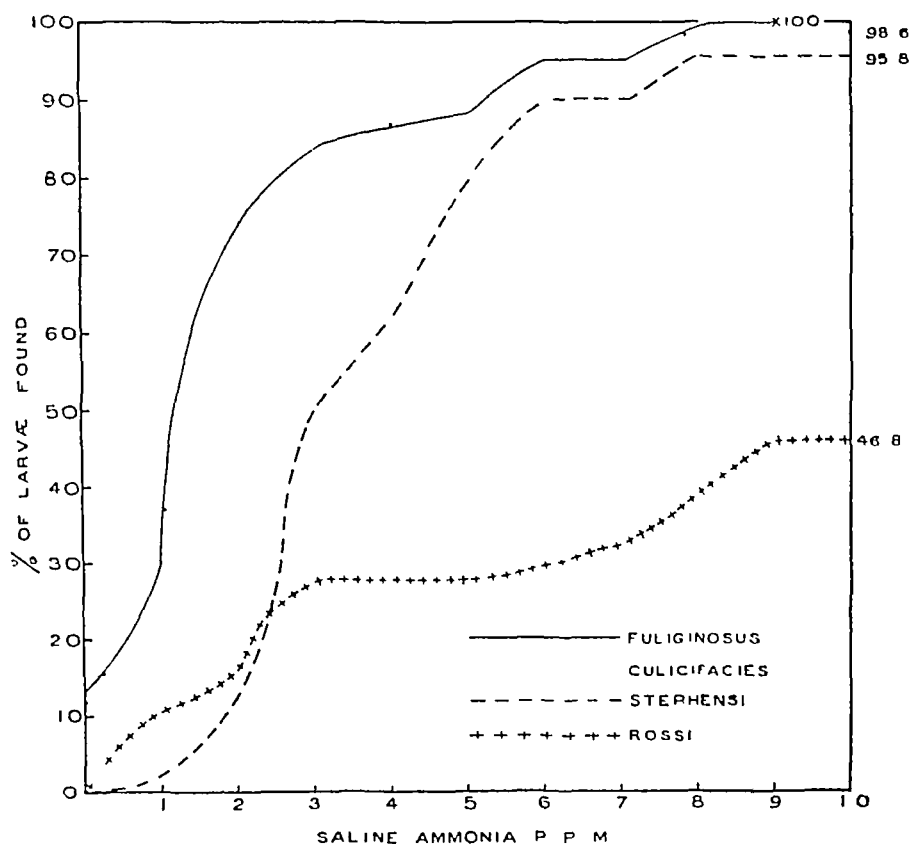


Fig 2 -Percentage of total catch of four species of *Anopheles* found at successive concentrations of ammonia up to 1 p p m

This discovery, as it turns out, is not altogether new. As long ago as 1903 Waddell, in a paper completely overlooked until 1927, showed that 1 part in 4,000, (250 p p m) of strong *liquor ammoniac* (sp gr 0.880) was absolutely fatal to mature larvæ, and computed that a dilution of 1 part in 30,000 (33 p p m) would be inhibitive to those newly hatched. These strengths are, of course, very much greater than those now found to be inhibitory (or even likely to exist) in nature. None the less, all credit, however belated, must be given to Waddell for his original discovery of the effect of ammonia on breeding.

Now *liquor ammoniac* ( $\text{NH}_4\text{OH}$ ) is not a compound likely to be found in Nature. Ammonium salts in natural waters would almost certainly exist as the carbonate or the chloride, possibly on sour soils as the sulphate. An experiment was therefore carried out to see how ammonium salts obtained their inhibitory effect. As on rare occasions (*vide* Table XI) larvæ of susceptible species had been found well beyond the normal toleration limit, it was thought that the effect might be produced on the eggs or very young larvæ, and that the few more or less mature larvæ which had been found in exceptional situations had started

life in water of normally low ammonia content which had risen to the high figures found through bacterial action subsequent to their hatching. The results of two experiments along these lines are given in Table XII.

The only food given in each experiment was a small piece of *Spirogyra*, as the addition of an algal culture would have vitiated the experiments owing to the extra salts introduced and the metabolic products of plant activity. The irregularities are not therefore to be attributed to the ammonium concentration. I think, however, that the experiments prove that, whatever the inhibitory effect may be, it does not operate by hindering the hatching of the eggs. We are

TABLE XII  
*Hatching of Eggs of A. stephensi in Ammonium Salts*  
*Experiment I*

Substance	Concentration p p m	No eggs	No hatched 24 hours	No surviv- ing 3 days	Day of last death	Instar died in
NH <sub>4</sub> Cl	100	6	6	3	15	IV
Do	2	6	6	5	17	IV
Do	1	6	6	6	13	?
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	100	6	6	5	16	IV
Do	2	6	5	5	15	IV
Do	1	6	6	6	15	IV
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	100	6	6	5	29	IV
Do	2	6	3	2	15	IV
Do	1	6	5	4	17	IV
Control 18 eggs, 15 hatched in 24 hours All dead 16th day						

*Experiment II*

					Shortening of life • Con- trol
NH <sub>4</sub> Cl	100	9	9	All died st 1-11, 8th day	17
Do	2	11	11	Do st 11, 16th day	9
Do	1	10	10	Last died st 11, 23rd day	2
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	100	9	9	All died st 1-11, 9th day	16
Do	2	11	11	Last died st 14, 30th day	
Do	1	11	11	All died st 1-11, 9th day	16
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	100	10	10	Last died st 14, 19th day	6
Do	2	11	11	All died st 11, 17th day	8
Do	1	11	11	All died st 1-11, 8th day	17
Control 1st larva died st 11, 25th day					

therefore left in ignorance as to how the inhibitory process operates, and I consider that the next great step forward in Anopheline control will be effected through its further study

Williamson (1928) has taken this investigation a step further, and has published preliminary results showing that the ratio of oxydised to ammoniacal nitrogen is a better test of the fitness of water for Anopheline breeding than is the pure measure of saline ammonia, and that the inhibitory effect is obtained when  $\frac{\text{oxydised}}{\text{ammoniacal}} \text{ N} < 1$ . So few exceptions, however, have been found in the method adopted by myself, and these probably explainable by subsequent ammonia rise, that the more complex ratio would have little extra value of itself. None the less this method throws out a hint as to how the process may work, by indicating that it is probably bacteriological, thus accounting for the failure of the experiments in Table XII, based on pure salts and distilled water.

A very close study of the nitrogen cycle in natural waters now becomes essential for further progress. Williamson's ratio means that the saprophytic bacteria engaged in reducing proteids to ammonia are acting at a greater rate than *Nitrosomonas* and *Nitrobacter* can carry on the oxygenation cycle. This point thus serves to explain my findings in Part I regarding the relationship between the presence of *A. funestus* (*listoni*) in ricefields and a high oxygen content, which means a low figure for ammonia.

*Albuminoid Ammonia*—Contrary to the expectations expressed in Part I, this has not served to explain anything, either directly or expressed as a ratio to saline ammonia.

These ammonia results, even though the mechanism of the action is still obscure, open up great possibilities. Waddell (*loc cit*) has suggested that the decrease of malaria in England has proceeded, not from better drainage, but from increased nitrogenization of the soil by the use of leguminous plants in agricultural rotations\*. Be this as it may (for there seems to be no evidence to show that *A. maculipennis* is less prevalent than it was in England, or anywhere in Europe for that matter, save where definite anti-larval measures have been adopted), we seem to have here a foresight of a method of control based on natural factors. The pollution of waters with animal manures is an obvious method, which I believe has been tried practically in the United Provinces by Col Fry, I M S, A D M S, Eastern Command. Such a method would not, of course, be of general application in view of the other equally important diseases which such a method would enhance. Its action against malaria is to substitute *rossi* for carrier species, as may be seen in any *culicifacies* pool fouled by the excreta of herbivora.

It may be, again, as Waddell suggests, that the final solution of malaria control will come from improved methods of agriculture, or finally, as suggested

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\* A much more probable reason in any case than the recent statement in the lay press by Sir William Willcox, which has created so much interest in India, that the reason is the inhibition of parasite development in the adult due to the imbibition of clover honey.

by myself (Senior-White, 1928b), by the employment of a bacteriophage to inhibit the growth of *Nitrosomonas*, thereby raising the ammonia content of waters to a lethal figure. In any case, the future appears full of promise.

## V CONCLUSIONS

1 It is useless to continue the study of Plankton in connection with mosquito breeding unless systematists capable of determining specifically all the organisms found are available in connection with the research. The mere generic identification of algæ has not yielded results of any value.

2 The study of the following physico-chemical factors has proved to be useless in connection with mosquito breeding: pH, conductivity, carbonates and albuminoid ammonia.

3 'Residual pH,' phosphates and dissolved oxygen may still be studied in the hope that they will yield results of value.

4 Saline ammonia is inhibitory to *Anopheles* breeding, save in the case of the *rossi*-group, in amounts exceeding one part per million.

5 The evaluation of nitrite and nitrite factors, hitherto unstudied, are required, to correlate with saline ammonia values.

6 The Winkler process for dissolved oxygen is useless in the presence of nitrites, and requires modification for use in such waters.

7 It has been impossible to confirm Hodgson and King's values for optimum and limiting temperatures in *Anopheles* breeding. Different species are shown to have apparently different optima, and much further work on the subject is required.

8 The confirmation of the discovery of the inhibitory effects on breeding of very small amounts of ammonia opens up, via several routes, the practical possibility of biological control.

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Fig 1



Fig 2



#### EXPLANATION OF PLATE VI

- Fig 1 Roshanara Gardens Tank, Delhi The laboratory attendant on the left of the picture is standing beside the routine examination spot
- „ 2 Irrigation Channel in Disused Land, Delhi The laboratory attendant is standing by the routine examination spot
- „ 3 The Riveram Bela Area of Delhi, flooded by the Jumna River Samples drawn from margin of flood water according to its position

#### EXPLANATION OF PLATE VII

- Fig 4 Roadside Borrow Pit, Delhi Showing levelling up of Bela with City  
rubbish in background
- „ 5 The Shahji Tank, Delhi City Sampled only when foreground area  
was flooded

PLATE VII

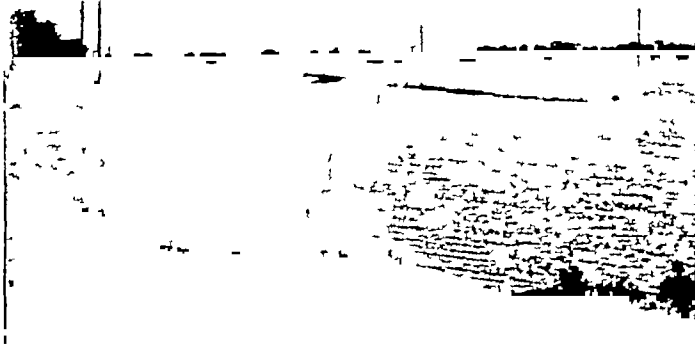


Fig 4



Fig 5



# STUDIES OF THE EFFECTS OF ANTIMONY SALTS

No 2

## THE EFFECT OF AMINOSTIBUREA ON CASES OF KALA-AZAR

BY

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RAM TARAN SEN, DPH (Camb),

AND

C DAS

[Received for publication, February 6, 1928]

Of recent years many preparations of antimony have been placed on the market for the treatment of kala-azar and, among others supplied us to test if it was any use, was one manufactured by the Union Drug Co of Calcutta, which they called Aminostiburea. The Company stated that the drug was an organic compound of antimony similar to some of the other better known compounds of antimony but with a different molecular composition, and they suggested it might be found a valuable drug in the treatment of kala-azar.

After testing the drug on animals to obtain the minimal lethal dose per body weight of various animals, we decided to try the drug on cases of kala-azar.

The drug is a fine pinkish brown coloured powder issued in glass ampoules. It dissolves readily on the addition of water into a transparent clear pinkish solution.

The dose we started with was 0.05 gm on all cases. This dose during the course of the treatment of each patient was gradually raised to 0.2 gm and in two cases to 0.3 gm without toxic symptoms. The drug was administered intravenously every second day during the treatment. In all cases the drug was tested on, we first punctured the spleen and examined slides of the puncture material obtained for Leishman-Donovan parasites of kala-azar, and also made cultures on NNN media and incubated for the flagellate form of the parasite. Only positive cases on the slides as well as the cultures were tested with the drug. All patients under treatment had this procedure carried out on them at least three times, once to prove the patient was suffering from kala-azar, a second time during the course of the disease to test progress with this unknown drug, and at least once more to prove whether the patient was cured or not.

No patient was considered cured unless —

(1) No parasites were found on the slide from the spleen puncture

(2) No parasites were found on culture in NNN media from repeated examinations of the cultures up to 10 days Four culture tubes were made at each examination, two of which were kept at 22°C and two at 26°C

The slides examined direct from spleen puncture were divided into four groups —

(a) Those in which the parasites were found in every field of the microscope These are shown in the tables as four plus

(b) Those in which the parasites were numerous but not in every field These are shown as three plus

(c) Those in which the parasites were scanty These are shown as two plus

(d) Those in which the parasites were very scanty only one or two being found in a whole slide These are shown as one plus

The formolgel test was carried out on every case, rather with the idea of checking the test than relying on it This test was divided into three groups —

(A) Complete typical gelification in 5 minutes

(B) Complete typical gelification in half an hour

(C) Complete typical gelification in 24 hours

This is shown in the tables with the plus and minus signs beginning from the left

A large proportion of these cases nowadays reaching the Pasteur Institute and Medical Research Institute, Shillong, Assam, have already received some treatment in the plains of Assam, and are either failures of treatment there, or else the patients are not satisfied that they are being cured by the treatment they are receiving, and pay their own way up to the Institute at Shillong The patients only know the name of the drug they have been treated with and the number of intravenous injections they have been given This is noted in the tables below as so many injections of T E or U S, T E meaning Sodium Antimony Tartrate, known locally as tartar emetic, and U S, Urea Stibamine, the two drugs in common use in Assam It is impossible to be certain of the amount of drug administered before they arrive at the institute

The duration of the illness is noted in the table below

The amount of drug administered, in this case, Aminostiburea, before cure is obtained is noted in the table

The number of days the patient is in hospital before a cure is obtained is also noted

Weight on admission and discharge is shown as follows —

$\frac{112}{119}$ , meaning weight 112 lbs on admission and 119 lbs on discharge

The size of the spleen is shown in the table as follows —

$\frac{\frac{1}{2} \text{ rt} \times 2 \text{ below}}{1 \text{ lt} \times 1\frac{1}{2} \text{ above}}$







$\frac{1}{2}$  rt = One half finger's breadth to the right of the middle line

× = by

2 below = Two finger's breadths below the umbilicus

The figures and letters above the line mean 'on admission', those below the line mean 'on discharge'

1 lt ×  $1\frac{1}{2}$  above = One finger's breadth to left of middle line by  $1\frac{1}{2}$  finger's breadth above umbilicus The size of the liver is shown in the tables as follows —

Palp = Palpable below the ribs under the costal arch

2 fb = 2 finger breadths, ditto

The letters and figures above the line, represent the condition 'on admission,' those below the line 'on discharge'

Hb = Hæmoglobin

R B C = Red blood corpuscles per cubic millimetre

W B C = White blood corpuscles, ditto

Other diseases found are usually worms, including hookworm, round-worm, *Gastrodiscus hominis*, *Fasciolopsis buski* and whip-worm These worms are all very numerous in Assam One patient passed 754 *Gastrodiscus hominis* in one stool and another over 100 *Fasciolopsis buski* in a single stool In both cases Thymol, 40 grs, had been administered as it had been found the most effective drug for the removal of these parasites

Eighteen cases in all were treated with aminostiburea and in all cases the patients were cured The minimum dose for a proved case of cure was 1.5 grms

The maximum was 6 grms and the average was 2.99 grms

Most of the cases had had the disease a long time before they arrived for treatment, the average being a year before they came for treatment One batch of the drug supplied showed slightly toxic symptoms in 3 out of 5 cases, and animals injected showed the same toxic symptoms, but a change to a fresh batch caused no repetition of the symptoms and no serious results occurred from the apparently slightly toxic action of that particular batch

We consider the drug a valuable addition to the drugs already discovered which show a curative action on kala-azar.



# EXPERIMENTS WITH VITEX PEDUNCULATA IN THE TREATMENT OF KALA-AZAR

BY

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AND

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[Received for publication, February 6, 1928 ]

VITEX PEDUNCULATA is a plant common in the forests of Assam, Bihar and Orissa, and is said also to be present in the forests of Bengal and other parts of India. It has a great reputation among the local inhabitants for curing many diseases especially malaria, blackwater fever, influenza and latterly kala-azar.

Knowles and Gupta (1924) investigated the question of its curative powers on malaria cases and proved that its curative powers for malaria were nil.

We decided to test the question of its powers in the cure of kala-azar, more especially as one of the acting civil surgeons in Assam reported that he had had excellent results with it, amounting in his opinion, to a cure. Any reputed cure of this terrible disease which kills so many thousands every year in Assam, Bengal and Madras and has in the past, before the advent of the treatment of this disease with antimony salts, depopulated whole areas of the country, is well worthy of a trial.

The local inhabitants have great faith in this remedy as, in common with people in other parts of the world, they believe that wherever a disease is found there the antidote will also be found growing in the plant life around, if one can only find it.

Two methods of obtaining the drug for use in kala-azar are in common use —

(1) A decoction made by 1 oz. of the leaves, or bark of the finer twigs, or bark of the root, in 40 ozs. of water, boiled slowly down to 15 ozs. and strained through muslin.

Dose—1 oz. rising to 4 ozs. three or four times a day.

(2) Infusion—4 ozs. of the leaves, or bark of twigs or roots dropped into boiling water which is allowed to continue boiling for 10 minutes and then allowed

to stand before the resulting fluid is poured off when required, the strength thus slowly increasing

Dose—1 oz three or four times a day

We tried the drug on two cases with the decoction method and two by the infusion method after first obtaining the consent of the patients. The method of testing whether the disease was present was by spleen or liver puncture, direct smear of the material obtained on a slide and also culture in NNN media

Spleen puncture was carried out at least three times in every case, once during the course of the treatment and finally at the end of the experiment. The results are shown in the table

These results are interesting. The cases were early cases without much previous treatment. The first two were treated with the decoction, the second two with infusion

(1) In most cases there was a distinct gain in weight, the average being 5 lbs

(2) Their general appearance of health was improved

(3) There was very little fever if any while under treatment with vitex pedunculata but —

(1) No case was cured with vitex pedunculata whether made into a decoction or infusion

(2) In every case the number of parasites present in the spleen increased steadily and also the size of the spleen

(3) They were all cured with urea stibamine though considerably larger doses than usual had to be used of this drug to procure a cure after the delay of treatment caused by the administration of the vitex pedunculata. We considered that vitex pedunculata had some effect on the patients by improving their general condition. This improvement is the probable reason why curative powers have been attributed to this drug by the local inhabitants. The drug, however, had absolutely no curative effect on kala-azar itself as the parasites increased steadily throughout the treatment until we considered we were not justified in risking the lives of patients by continuing the test

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*Ind Med Gaz*, Sept

TABLE

No	Age	Previous Treatment	Duration of Disease	Length of Treatment with Vitex	Weight	Spleen Size	Spleen Punctures	Result	Subsequent Treatment and Results
1	21	4 injections T E	3 months	31 days	104 lbs 115 "	2 f b below umbil	+ + + + + + + + + + + + +	Not Cured	U S 2 85 grms Cured
2	14	13 injections T E	7 months	32 days	53½ lbs 58 "	5 f b below ribs 5 f b below ribs	+ + + + + + + + + + + + +	Not Cured	U S 4 2 grms Cured
3	27	2 injections U S	3 months	38 days	117½ lbs 117½ "	Palp 4 f b below ribs	Liver puncture + Spleen puncture + + + + + + + + +	Not Cured	U S 3 25 grms Cured
4	24	2 injections T E	3 months	40 days	116 lbs 124 " 120 "	1½ f b below ribs 5 f b below ribs	+ + + + + + + + + +	Not Cured	U S 3 70 grms Cured

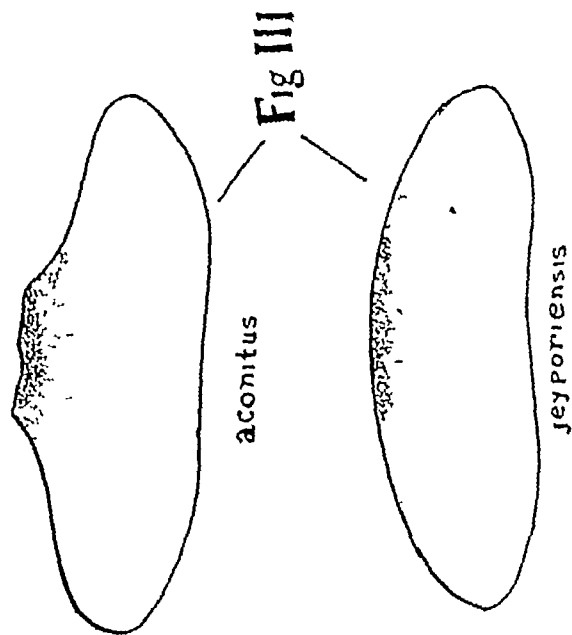
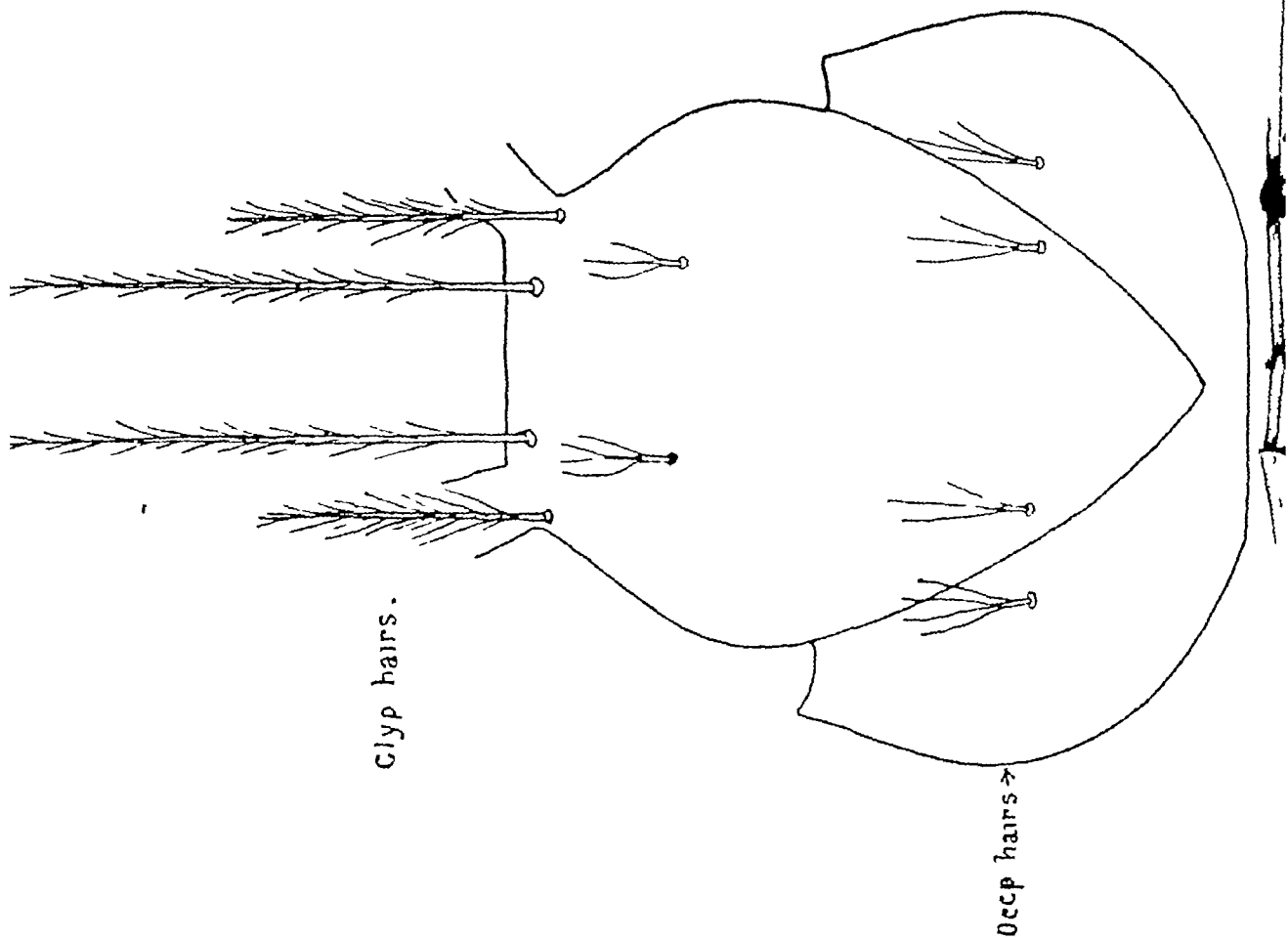


Fig II

# THE LARVA OF *A JEYPORIENSIS* JAMES

BY

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[Received for publication, February 10, 1928 ]

As James and Liston's description of *A jeyporiensis* larva in their 'Anopheline Mosquitoes in India' is not complete in some respects and their figure of this larva (Plate VIII, fig II) not very accurate, this short description is now submitted. This seems to be all the more necessary as the larva may sometimes be confused with that of *A acontus* or *A philippinensis*.

## THE LARVA OF *A jeyporiensis*

It is medium sized and usually brown

### Head —

#### Clypeal hairs —

Ant Int—stout with pinnate branching—which is much stronger and more regular than in *A philippinensis* or *A acontus*

Ant Ext—very stout with strong thick branching

Posterior—a long tuft of three to five branches arising on a stem arising from the basal papilla

Occipital hairs—Both branched as in fig I (Plate VIII)

The mentum has seven teeth

Thorax—All the thoracic hairs are deeply pigmented grey. Palmate hair is well-developed with about 12 leaflets devoid of filaments

Abdomen—Palmate hairs are well-developed on all the segments. The blade of each leaflet has a steeply serrated extremity with a short and sharp filament (see Plate VIII, fig II). There are 16 to 20 leaflets in each palmate hair on the mid-abdominal segments

The dorsal plaques are larger than usual and look almost like those of *A. acontus-funestus*, but with the following points of difference—(a) smaller in size, (b) the mid-anterior parts is not raised like that of *acontus* and is diffusely pigmented, (c) posterior margin tends to be more concave (see Plate VIII fig III)



The following table shows the diagnostic points of *jeyporiensis* from *acomtus* and *philippinensis*

	<i>A jeyporiensis</i>	<i>A acomtus</i>	<i>A philippinensis</i>
A Clypeal hairs —			
Ant Int	Thick pinnate branching	Thin pinnate branching	Thin pinnate branching
Ant Ext	Strong thick pinnate branching	Thin pinnate branching	Plumose branching
Post.	Long tuft branches arising from a stem	Short tuft branches arising close to base	Thick tuft of more than six branches arising from 2 or 3 stems
B Dorsal plaques on abdomen	Large size with convex anterior margin, and concave posterior	Large size with raised anterior margin	Small, oval
C Palmate hair on thorax	With about 12 leaflets and no filaments	Most developed with 16—18 leaflets	Well-developed with 12—16 leaflets
D Palmate hair on abdomen	Leaflet has steepled serrated extremity with a short and sharp filament	Cup-shaped extremity, filament long and sharp	Ordinary shape with long filament

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# A NOTE ON THE LARVA OF *ANOPHELES PHILIPPINENSIS* LUDLOW, 1901, AND ITS DIAGNOSIS

BY

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[Received for publication, February 10, 1928]

PURI (1927) described the larvæ of two mosquitoes caught in Western India which he thought to be *philippinensis*. Subsequently, he received some *philippinensis* larvæ from Cachar which were indistinguishable from these. Their characters were as follows — 'Internal anterior clypeal hairs longer and stronger than in *pallidus* on the distal half thickly plumose posterior clypeal hairs dividing at their base into 7 to 10 branches Internal occipital hairs with 3 to 4 branches Mentum with 9 teeth (like *pallidus*) Filament of palmate hair less than half the length of those found in *pallidus* The differences from *pallidus* exhibited by these specimens were slight, but marked'

While malaria-surveying some tea gardens in Upper Assam (Dibrugarh area) and in Cachar (North Cachar hills) last year, I examined a very large number of *A philippinensis*, both adults and larvæ, and my conclusion is that the larva of *A philippinensis* can easily be distinguished from any other anopheline larvæ except those of *A pallidus* Theobald, from which, however, I think, it constantly differs in the form of the abdominal palmate hairs

## LARVA OF *A philippinensis* LUDLOW, 1901

A brown larva of medium size

Head—*Clypeal hairs*

Ant Int—Pinnately branched distally along three-fourths of its length

Ant Ext—Strong plumose-branching, like that of *A fuliginosus* Giles

Posterior—A tuft of six or more twigs arising from two or three short stems coming out of the basal tubercle (*see* Plate IX, fig 2)

*Occipital hairs*

Internal—A tuft of several twigs *always* arising from two or three short stems formed by splitting up the basal stalk (*see* Plate IX, fig 2)

External—A long tuft of five or six branches

*The mentum*—Has seven teeth like others of this group

*Thorax*—A palmate hair well-developed with 12 to 16 leaflets devoid of filaments

*Abdomen*—Leaflets of the mid-abdominal palmate hairs have sharp filaments about half the length of the blades

(Cf *A. pallidus* Theobald, which has long narrow filaments about equal in length to the blades)

In the absence of any figures in Puri's paper it is not quite certain to what extent his description of his specimens correspond with mine except in one point, viz, the mentum, and perhaps in the internal occipital hairs. I have invariably found the mentum to have 7 teeth in this species, while Puri gives it (and *pallidus*) 9. Perhaps his Yellapur specimens were not *philippinensis*, they ought not to be from there\*.

#### DIAGNOSIS OF *A. philippinensis* LARVA

The table gives the various diagnostic points of *A. philippinensis* from certain other species and the figures (1, 2, 3) (Plate IX) from those species with tufted external clypeal hairs. I have found that the mentum is of no use in the diagnosis of these species, as they all have 7 teeth to it.

It will be seen from figures 1 and 2 (Plate IX) that Puri's statement that the larvæ of *fuliginosus* and *pallidus* are very similar is only relatively true. The posterior clypeal hairs and internal occipital hairs are, I think, quite and invariably distinct. The latter point Puri himself mentions. I do not agree that *pallidus* larva has 9 mental teeth while *fuliginosus* has 7.

Dr Strickland's diagnosis of *jamesi* from *fuliginosus* (1925), and also the note in Strickland and Chowdhury (1927) on the same subject must be read as the diagnosis of *philippinensis* from *jamesi* in the light of the present research.

In conclusion I beg to thank Dr C Strickland, M A, B C, F R G S, Professor of Medical Entomology, School of Tropical Medicine, Calcutta, for his help in the matter of this paper.

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- |  |  |
|--|--|
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\* Dr Carter has sent some specimens of *philippinensis* larvæ to Dr C Strickland who has kindly let me see them.

## Differences in the larvæ of Anopheles

Structures	<i>fuliginosus</i> , Giles	<i>*philippinensis</i> , Ludlow	<i>jamesi</i> , Theobald	<i>† pseudojamesi</i> , Strickland and Chowdhury	<i>maculipalpis</i> , Theobald
A Clypeal hairs { Ant Int Ant Ext Posterior	Strongly frayed	Strongly frayed	Strongly frayed	Thinly frayed	Thickly frayed
	Dendriform branching	Dendriform branching	Dendriform branching	Strong fraying	More thickly frayed
	Tuft of 3, 4, or 5 twigs arising close to the root in most cases Simple and bifid may also be seen	Tuft of more than 6 twigs arising from 2 or 3 short stems coming out of the root	Simple, bifid or trifid — up to 5 twigs but always arising on a distinct stem This hair is longer than that of <i>A. fuliginosus</i>	Simple always	Simple always
B Occipital hairs { Internal External	Simple or bifid	Characteristically branched the branches arising from two or three stems coming out of the root	Simple	Simple	Bifid
	Branched	Branched	Branched	Simple	Branched
C Abdominal palmate hairs (i) On 1st segment { On 2nd segment   On 3rd segment (ii) Filament of a leaflet	Well developed, but small	Well developed, but small	Rudimentary	Rudimentary	Well developed
	Well developed	Well developed	Well developed	Rudimentary	Well developed
	Well developed	Well developed	Well developed	Well developed	Well developed
	Long and sharp	Long and sharp	Long and sharp	Short and sharp	Short and blunt

\* *A. pallidus* larva differs from *A. philippinensis* only in the form of the abdominal palmate hair

† *A. ramisayi* Covell, according to Puri, is generally similar, but I have not been able to obtain any specimen  
hair two-thirds the length of the internal, while in *pseudojamesi* it is about a half

He, however, makes the external clypeal

# PLATE IX

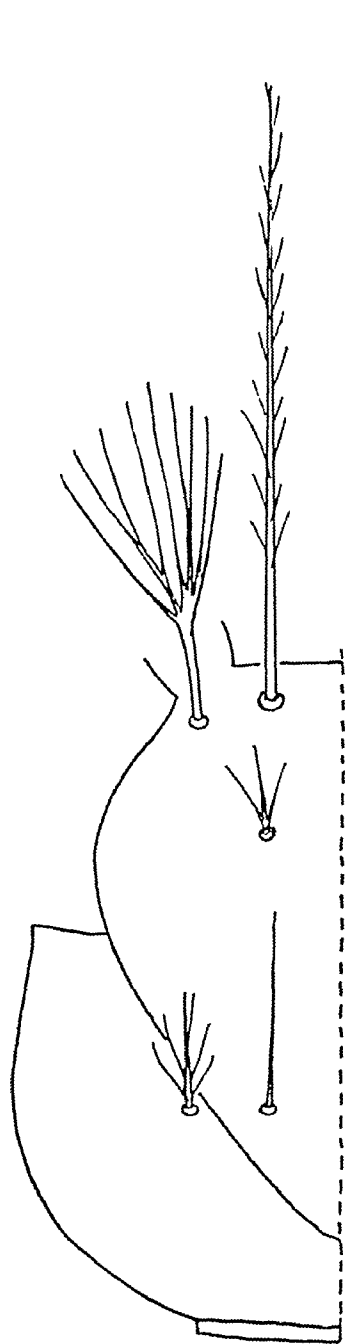


Fig 1  
*A. fuliginosus* Giles

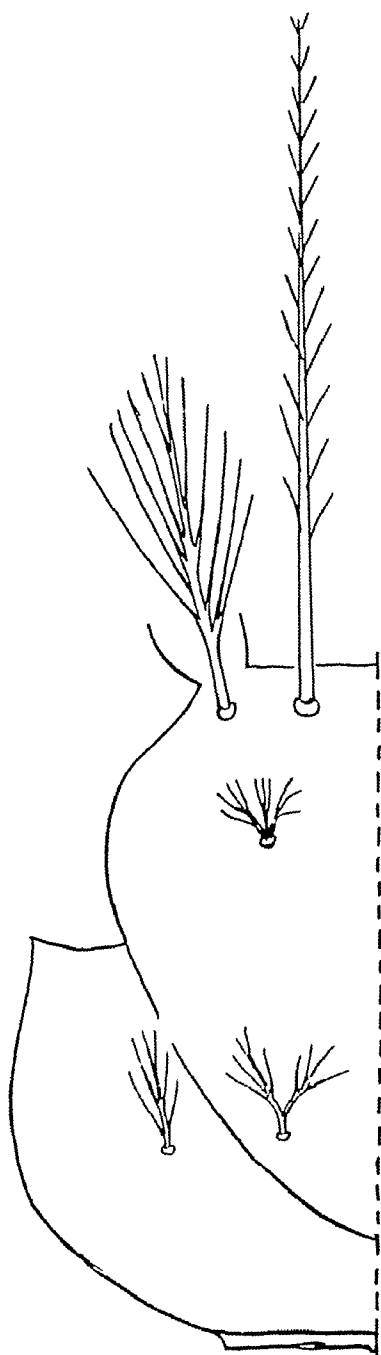


Fig 2  
*A. pallidus* Theobald  
or  
*A. philippinensis* Ludlow

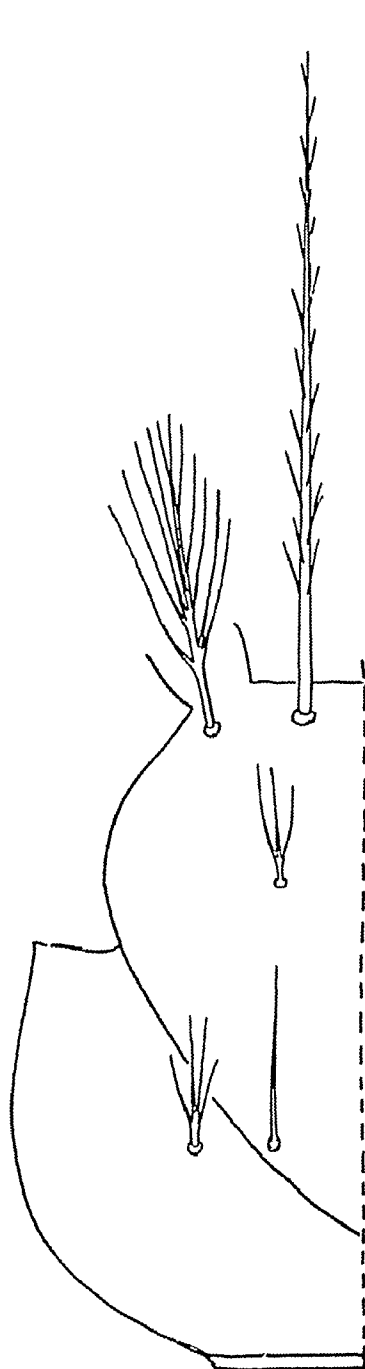


Fig 3  
*A. jamesii* Theobald

# ANOPHELES PHILIPPINENSIS AS A NATURAL CARRIER OF THE MALARIA PARASITES IN BENGAL

BY

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[Received for publication, February 25, 1928]

ALTHOUGH *Anopheles philippinensis* Ludlow has so far been recorded from only two localities in Bengal, namely, Jalpaiguri and Chittagong (Covell, 1927a), there is no doubt that this species is fairly common in Bengal. As the observations both of myself and of Mr M O T Iyengar, Entomologist of the Bengal Public Health Department, show, it has a wide distribution in the districts of Bengal. The sparsity of previous records is explained by the possibility of this species having been mistaken for *A. fuliginosus* which it closely resembles. The three species, *A. pallidus*, *A. fuliginosus* and *A. philippinensis* are closely allied to each other and are frequently mistaken for one another. All three species occur in Bengal and the principal points of difference between them are given below (after Christophers, 1924)

Wings darker, pale interruptions on costa very narrow, vein 5 extensively dark, basal portion of costa mainly dark. Joint between 1st and 2nd tarsal segments of hind legs conspicuously picked out with white, no pale scales on ventral aspect of abdomen, scale tuft on ventral aspect of 7th segment not very conspicuous, few or no scales on prothoracic lobes

*fuliginosus*

Wings lighter, pale interruptions on costa in apical half of wing not narrow, vein 5 extensively pale, base of costa pale with small dark spots

White scales present on ventral aspect of most of the abdominal segments, tuft of scales on ventral aspect of 7th abdominal segment very conspicuous, scales present on prothoracic lobes, joint between 1st and 2nd hind tarsi never picked out with white

*pallidus*

No white scales on ventral aspect of abdomen, scale tuft on ventral aspect of the 7th abdominal segment present but not so conspicuous as in *pallidus*, joint between 1st and 2nd hind tarsi usually picked out in some degree with white

*philippinensis.*

So far as I have been able to refer to previous literature, I find that *Anopheles philippinensis* has not been found to be infected with malaria parasites either in laboratory experiments or in nature. In fact it has been classed as a non-carrier species. Ludlow (1914) says of this species, 'Relation to malaria unknown'. Covell (1927a) says 'There is no evidence that this species is concerned in the transmission of malaria, and, in any case, its numbers are probably too scanty for it to play an important part in this connection in India'. Again, Covell (1927b) in a subsequent paper says 'This species is not generally considered to be a carrier of importance. Specimens provisionally recorded as this species were, however, found infected in nature in Burma by Feegrade (1926)'. This reference to Feegrade's results is an unpublished official report by Feegrade, which I have not been able to consult.

It is clear, therefore, that very little is known of the infectivity of *A. philippinensis*. The finding by me at Krishnagar, of naturally infected specimens of this species, both with sporozoites as well as zygotes is therefore of much interest. The specimens were carefully examined and the species determined before dissection and there is no doubt of their identity.

The following dissection record consists of specimens collected from hyperendemic villages in the vicinity of Krishnagar (Nadia district, Bengal), where the spleen rates of children were found to be over 90 per cent. The specimens were dissected at the Malaria Research Laboratory at Krishnagar.

*Record of dissection of Anopheles philippinensis*

Period of dissection	Number dissected	Zygotes	Sporozoites	Total number found infected	Percentage
October 1927	125	1	1	2	1.6
November 1927	98	2	4	5	5.1
TOTAL	223	3	5	7	3.1

During October 1927, out of 125 definitely identified specimens of *A. philippinensis* 2 were found infected, giving an infection rate of 1.6 per cent. During November, out of 98 specimens, 5 were found infected, giving an infection rate of 5.1 per cent. Taking the two months together, the rate comes to 3.1 per cent. The figure is high, apparently influenced by the hyperendemicity of the villages from which these specimens were collected. These figures are interesting as they are nearly the first definite records of infectivity of *Anopheles philippinensis* in nature. I am hoping to extend this work during this year and I expect to carry on more intensive work on this species during the ensuing season.

I am much indebted to Mr M. O. T. Iyengar for the help I received from him in connection with the identification of specimens sent to him.

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# ANIMAL EXPERIMENTS AND SPRUE

BY

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(Being Part II of the Sprue Investigation at the Haffkine Institute.)

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MOST of the strains of monilia used were recently isolated from clinical cases of sprue and were mostly of the species *M psilosis* (Ashford), some were irregular strains of *M psilosis* and others were *M krusei* (Cast) a type of wild yeast. Strains of *M psilosis* from Colonel Ashford's own laboratory were also used as well as certain yeasts from the National Collection of type cultures from the Lister Institute, London

## *I The result of a single peritoneal injection of yeasts into animals*

Ninety-one different animals including monkeys, guinea-pigs (chiefly), white mice and rabbits were injected. The doses were generally large, 3 c.c. of a rich emulsion from a maltose agar slope or growths in fluid media were used. Only four animals died as a result of a single inoculation. Some were killed at varying intervals after the inoculation, whilst others were allowed to recover and were returned to stock.

The details of the four fatal cases are as follows —

(1) Animal—guinea-pig, type of yeast—*M psilosis* (Ashford) (fæcal strain from his own laboratory), dose 5/6th of a 48 hours Sabouraud's medium slope, died on 2nd day

(2) Animal—guinea-pig, same strain as (1) after passing through three animals, dose 5/6th of a 48 hours slope, died on 2nd day

(3) Animal—rabbit, inoculated with *M krusei* (Cast) L I P M 2/5th of a 24 hours agar slope, died on 5th day

(4) Animal—guinea-pig, *M psilosis* isolated from stools of a healthy man, 5 c.c. of a four days old fluid culture, died on 2nd day

*Post-mortem appearances of these fatal cases*—Except rabbit No 3 (in which local changes were slight), the animals showed a condition of acute plastic peritonitis with exudate of blood-stained lymph, the adhesion of coils of intestine and the presence of numerous yeasts in the exudate. In some cases the yeasts were recovered from the heart blood and viscera but no focal deposits were noted in the viscera at this stage. Pericardial and pleural exudates were sometimes noted.

Those animals which were killed at different periods after inoculation showed the various stages of plastic peritonitis, as described in above paragraph, terminating in those which lived longer in the presence of fibrous adhesions or of thickening of the peritoneal covering of organs. In some cases small foci resembling chronic abscesses containing inspissated pus or caseous material was all that remained of the infection. Such pus frequently contained free or phagocytosed yeasts generally in a living condition. The full details of these experiments, including the result of visceral culture, are given in the accompanying Protocol I, which shows that blood infection is not uncommon in the early stages after inoculation but generally disappears if the animal survives longer than 3 or 4 days.

## *II Experiments to show the course of the disease in a series of animals inoculated with the same dose of the same strain of yeast at the same time*

Ten guinea-pigs of approximately the same size were taken and each given 5/6th of a Sabouraud's slope of Ashford's faecal strain of *M. psilosis*. One was killed each day as long as they lasted and the local and cultural results noted (*vide* Protocol I, Nos 28—37). It will be seen that a monilia septicæmia was present up to the third day, that yeasts were recovered from visceral culture till the 4th day and after that they were found by culture of the peritoneal fluid up till the 10th day. The local stages of peritonitis were graded from the first day when they were severe till the 10th when small caseous nodules or adhesions were present and all signs of general inflammatory reaction had disappeared. None of the ten animals died after injection.

## *III The result of multiple intraperitoneal injections*

Fourteen experiments of this nature were performed (for details *see* Protocol II). Five of the animals died and a condition of plastic peritonitis with adhesions was found in all. Monilia were generally recovered from the viscera after death showing that a monilia septicæmia had occurred.

Four animals were sacrificed at varying intervals after the last injection and at the autopsy plastic or adhesive peritonitis was found.

Five animals which showed no symptoms after the injections recovered and were returned to stock.

*Summary*—Following the intraperitoneal injection of yeasts from sprue and other sources the majority of animals recover without general symptoms. Those

that die or are killed show peritonitis of varying degrees and monilia are generally capable of being recovered within a few days of the injection in a living state from the blood or from the viscera

#### IV *The result of a single intravenous inoculation*

Eleven experiments were done on rabbits and one on a monkey. Four died, six were killed at varying periods after the injection and two were allowed to recover. One notably toxic strain (Gomez monilia) (originally recovered from the duodenal contents during life of a case of severe sprue) was used, but other strains isolated from sprue faeces were found to be relatively atoxic.

Gomez monilia was remarkably toxic to rabbits and all animals inoculated with it by the intravenous route died or were killed when in a moribund state. Death was preceded by local spasms, e.g., torticollis or general convulsions with air hunger and respiratory failure suggesting uræmia. In all cases the kidneys were found to have borne the brunt of the attack—the cortex being generally riddled with minute focal necroses so much so that in severe cases the whole cortex of the kidneys was dotted with small pin point infarcts. Monilia could be recovered in abundance from these areas and sometimes from the heart blood, liver, spleen and lungs. Microscopic examination showed abundant growth of yeasts in budding and in mycelial forms in these areas of necrosis and the surrounding areas of the kidney substance were destroyed by necrotic and inflammatory changes. Yeasts were sometimes discernible in small groups in the liver and other viscera, but these were not accompanied by the same degree of local necrosis as in the kidney. The condition in the kidneys was doubtless the result of infarction of the smaller vessels with subsequent growth of monilia, resulting in widespread destruction of the secreting substance of the kidney and death with symptoms resembling uræmia.

In some cases, however, the death of the rabbit followed within 24 hours of the injection, presumably of toxæmia. The kidneys in such cases were inflamed and in a state of coagulative necrosis without the demonstrable presence of yeasts. In the few cases which lived for some time the kidney lesion had begun to clear up and yeasts were no longer found in a living state in the necrotic areas. Plate I shows the changes in the kidney.

Fig 1 shows the life size appearance of a badly diseased kidney

Fig 2 shows a low power view of the cortical lesion

Fig 3 shows a high power magnification of a necrotic area and the presence of abundant growth of monilia

(For further details see Protocol III)

#### V *The result of multiple intravenous injections of monilia from various sources*

This series included 27 animals, of which five died after the injections and 22 were killed at different stages following the injections\*. Of the five which

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\* The toxic strain *M. psilosis* 'Type Gomez' was not used in these multiple injections as this strain always kills on the first injection.

died one had received three injections of *M krusci*, two of *M albicans* (Crak) and two of a strain of *M psilosis* isolated from a sprue case in Bombay. In four out of the five the monilia were recovered from the blood or viscera after death. In those animals which were killed the monilia used had generally been isolated from sprue cases but some were of the *M krusci* class. Monilia were sometimes recovered from the blood or viscera after death but in the majority they were not found on culture. At the necropsy in most cases minute necrotic foci or infarctions were found in the renal cortex. The alimentary tract was always scrutinised and in no case was any disease found nor were there any clinical signs during life remotely suggesting sprue.

(Details of these cases are shown in Protocol IV.)

#### *VI Feeding experiments*

Six experiments were made in which the most toxic strain of *M psilosis* (Gomez) were fed to two rabbits, two white mice and two guinea-pigs. The doses were large and a rich culture was given by the stomach tube or spread on food. The animals were kept about three weeks but no diarrhoea or other intestinal signs were observed. The animals were killed, their alimentary tracts were found to be healthy and monilia were not recovered by visceral or blood culture in any case.

(See Protocol No. V.)

#### *VII Miscellaneous experiments*

A few experiments were made either by combining two routes of injection, intravenous and intraperitoneal, or by intrathoracic or intralingual injection. The intralingual injections produced no local or general infection differing in this respect from Ashford's experiments.

#### *VIII Experiments to ascertain whether the virulence of a strain of monilia is enhanced by ten passages in a succession of guinea-pigs*

Ashford's original strain of *M psilosis* was used, and this was injected by the peritoneal route into a guinea-pig. Two days later the animal was killed and the monilia recovered from the tissues grown for 24 hours on an enriching medium and a similar dose injected into a second guinea-pig. This was repeated on ten animals. No enhancement of the virulence was noticed as judged by the severity of the symptoms or by the appearances at the autopsy.

#### *IX The injection of duodenal fluid from cases of sprue into animals*

One of our experiments had shown that a monilia found in the duodenal fluid (together with an unrecognised bacillus) was highly toxic to rabbits by intravenous injection. We therefore injected the duodenal fluid from several cases in different stages of sprue into animals. Eleven experiments of this nature were performed. The duodenal contents (or bacteria therefrom) were given by the subcutaneous route in 8 cases and by the peritoneal route in three. Three of the former died but the condition present was not attributable to

monilia but to secondary infection of a bacterial nature. Nothing resembling sprue was noted in those which recovered (See Protocol VI)

### X Experiments with animals suffering from a deficiency of Vitamin C

Having failed to produce signs of sprue in normal animals, we attempted to do so in animals in a condition of incipient or pronounced scurvy. Monkeys, mostly *M. sinicus*, were the animals chosen for the experiment. The diet prescribed for the monkeys was kindly suggested by Lieutenant-Colonel R McCarrison and consisted of the following articles —

Boiled polished rice	100 grms dry weight
Ground-nuts	30 grms
Autoclaved milk	200 c c
Orange juice	2 c c
Butter	10 grms

The animals were kept on this until they showed a loss in weight and other signs of scurvy which generally took about six weeks to appear. The faeces of ten animals were examined for yeasts prior to the commencement of the experiment with the following results —

<i>M. psilosis</i> (Ashford)	found in 5 animals
<i>Cryptococcus</i>	„ „ 7 „
<i>M. krusei</i>	„ „ 2 „
No monilia	„ „ 1 „
Not examined	3 „

The results were briefly as follows —

- I Exp No 278, fed on sprue faeces. Died with signs of dysentery three months after first infected feed. Post-mortem signs of acute colitis. Monilia not recovered from viscera. Cultures of H B and liver were sterile.
- II Exp No 279, fed on sprue faeces. Developed mild dysentery but recovered after ten days' illness.
- III Exp No 280, fed on sprue faeces. Developed dysentery of severe type two months after first dose. Recovered on being placed on full diet.
- IV Exp No 281, fed on sprue faeces. Developed dysentery and died about three weeks after first dose. Autopsy showed acute colitis and proctitis. Cultures of H B spleen and liver were sterile.
- V Exp No 282, fed on cultures of *M. psilosis*. Developed dysentery about three weeks after first dose and was killed in an advanced stage of emaciation. Post-mortem showed signs of colitis. Cultures of H B and liver were sterile.
- VI Exp No 283, fed on cultures of *M. psilosis*. Developed dysentery and died about two months after first dose. Post-mortem showed acute colitis and proctitis. Culture of H B was sterile.
- VII Exp No 284, control, fed on ordinary diet. Remained healthy and returned to stock.

- VIII Exp No 285, control, fed on ordinary diet Remained healthy and returned to stock
- IX Exp No 286, control, fed on ordinary diet Died from causes unknown Did not show dysenteric signs during life, and after death the alimentary canal was found to be normal
- X Exp No 287, control, fed on ordinary diet Died from causes unknown Culture of H B was sterile

*Second series of monkeys under same conditions of vitamin C deficiency*

- XI Exp No 327, fed on cultures of *M psilosis* and (once) on saline suspension of sprue faeces Developed marked signs of scurvy Showed no evidence of dysentery during life Killed on account of advanced scurvy The large intestine was in a condition of mild inflammation with chronic thickening of the mucous and submucous coats
- XII Exp No 328, fed on suspension of sprue faeces Died suddenly after showing mild signs of vitamin deficiency (loss of weight) No signs of dysentery during life Post-mortem showed advanced state of subacute colitis Culture of H B was sterile
- XIII Exp No 329, fed on suspension of sprue faeces Died with signs of nutritional disturbance without intestinal symptoms Post-mortem showed subacute inflammation of large bowel
- XIV Exp No 330, control, fed on diet deficient in vitamin C Died with symptoms of scurvy in terminal dysentery Post-mortem showed acute inflammation of whole length of large intestine *Bacillus coli communis* was isolated from cultures of H B and spleen
- XV Exp No 331, control, fed on diet deficient in vitamin C Was killed in a condition of advanced emaciation with signs of scurvy without intestinal symptoms Post-mortem showed extensive inflammatory change in large bowel including perforating ulcer and abscess formation extending into substance of liver Culture of H B was sterile Culture of liver abscess showed Flexner bacillus
- XVI Exp No 332, control, fed on diet deficient in vitamin C Developed signs of scurvy and was killed when it appeared likely to die The large intestine showed extensive inflammatory changes as in the previous cases

After completion of the first batch of ten monkeys it was seen that out of the six which had been fed on a diet deficient in vitamin C all developed symptoms of dysentery during life and in four which came to autopsy there were signs of a varying degree of inflammation of the large bowel The four control animals showed no such signs We were tempted to infer that the infected feeds with sprue faeces or with monilia isolated therefrom were responsible for the changes in the alimentary tract

In the second series of six monkeys the three controls were kept on a vitamin C free diet and it was then found that they suffered equally severely from a

dysentery-like disease and the presumption is that the exciting cause of the disease was not the infected feeds but was in some way associated with the scorbutic state. In none of the animals which died were monilia found in the blood stream or in the viscera, but other pathogenic organisms were scarce.

Careful examination of the intestinal lesions were made by microscopic examination of the fresh scrapings and also by plating out for dysenteric or other organism. In one case only was an organism resembling *B. dysenteriae* (Flexner) isolated and in one or two others lactose non-fermenting bacilli of uncertain nature. The extensive dysentery-like lesions were presumably of a non-specific nature inasmuch as none of the recognised specific dysentery organisms either bacterial or protozoal could be demonstrated.

It may be noted that none of the animals showed any sprue-like signs during life, and as regards the purport of these experiments they were wholly negative unless the view be taken that sprue in man is represented by a dysentery-like disease in monkeys which is an assumption unsupported by valid evidence.

The relationship between vitamin C deficiency and inflammation of the alimentary tract is of great interest apart from the object of this paper and for this reason the writers intend to publish these experiments in a separate paper dealing with the association of intestinal disease with avitaminosis. The full details of the experiments with cultural results and histological changes are reserved for this paper.

#### THE LITERATURE ON ANIMAL EXPERIMENTS IN SPRUE

P. H. Bahr's (1915) (now Manson-Bahr) conclusions are as follows —

'Feeding experiments with cultures of sprue yeasts and those of the true *M. albicans* produced no ill effects in any animals, even though monkeys and rabbits were fed daily with large doses.

Subcutaneous injections produced localised abscesses in guinea-pigs, in the resulting pus many vacuolated and evidently defunct yeast cells could be seen microscopically.

Intraperitoneal injections of broth cultures differed widely in their effects. In monkeys constant injection of small doses over a long period produced no symptoms, in guinea-pigs a subacute peritonitis, and in rabbits localised peritoneal growths.'

Using the intravenous route on 26 separate experiments on rabbits he showed that definite toxic effects followed and ten rabbits died. The yeasts could be cultivated from the various viscera and the kidneys in particular were the seat of necrotic foci in which budding and mycelial growth of yeasts were demonstrated. In short his experiment yielded almost precisely similar results to those now described by us. Bahr's concluding remarks are as follows —

'Although the results of these numerous experiments must be regarded as somewhat indefinite they seem to indicate that the sprue yeasts possesses a certain degree of pathogenicity to rabbits on intravenous injection, a feature in which they agree with cultures of *M. albicans*, the thrush fungus, and that, though possessing the same morphological and cultural features, yet by these means they may be separated in the majority of yeasts 'from other sources'.



Ashford's (1916) conclusions are as follows —

1 The species of monilia recovered by me from now nearly one hundred cases of sprue, and apparently a new species from careful cultural and morphological investigations, is a pathogen for current laboratory animals by hypodermic inoculation

2 This species, which I will for the present call *Monilia X*, is ordinarily a low virulence organism

3 When recovered from a patient with sprue and promptly injected into certain laboratory animals, it generally produces their rapid death from a mycotic septicæmia

4 When grown for a long time and frequently transplanted the same organism which rapidly killed soon after isolation from a sprue patient seems to partially or completely lose its virulence

5 This virulence may be recovered by passage through susceptible animals, and even reach such a point as to sicken or kill these animals by continued feeding

6 Ordinarily an animal may not be killed by feeding *Monilia X* when first isolated from a sprue patient. Its virulence must be augmented by passage before uniform pathogenicity through feeding can be demonstrated

7 In such animals the symptoms depend on the part of the intestinal tube most affected, in the portion in which these monilia secure their first foothold

8 A certain number of animals exposed by feeding, rapidly die of a monilia septicæmia believed by me to be due to a sudden primary pneumonia and secondary septicæmia

9 Animals escaping this fate succumb more slowly to what seems to be a toxin developed in the intestinal tract by a localization of these monilia

10 Feeding experiments have resulted in the production of a stomatitis on two occasions and in the appearance of severe and long-continued diarrhœa in several occasions

11 *Monilia septicæmia* causes the necrotic areas in animal organs referred to as "white spots" macroscopically. This has been verified microscopically in kidneys, and presumed to be the same for all organs so affected. Such organs are highly congested, dark red, and friable

12 Localized in the skin, typical blastomycotic ulcers are formed whose characteristic is necrosis without pus formation. If an internal organ is attacked large colonies of monilia are seen which look like emboli. Intervening spaces are generally free from the organism. This explains a casual failure to obtain a successful culture from an evidently infected organ, and thus differs radically from a bacterial septicæmia

13 Microscopically, infected organs show large clumps of monilia surrounded by an intense inflammatory zone. I have never seen pus produced by monilia

14 In one guinea-pig presenting a severe stomatitis, sections of the affected zone revealed monilia in the midst of the muscular bundles below the subepithelial connective tissue. This may explain the tendency for sprue to

recur after an apparent dietetic cure the yeasts are starved out in the surface, the patient apparently recovers, and, later, the deep lying roots of the mycelia layer push out towards the surface and re-establish a surface growth with its consequences and a relapse

15 In experimental animals in whom mycotic septicæmia is induced by intraperitoneal injection, the lungs are the most grossly affected, after them the kidneys and microscopic examination corroborate the gross anatomy'

Dold (1917), on the other hand, produced lesions in monkeys, dogs and mice by injecting 'wild' yeasts with results similar to those from yeasts isolated from cases of sprue

Fleisher and Wachswiak (1923), referring to the yeasts recovered from sprue cases, conclude that 'the fact that these organisms fed to animals produced diarrhœa and death and that the organisms could usually be recovered from both the intestinal mucosa and the heart blood at autopsy, appear to confirm their etiological relationship to the disease in human beings'

Potter (1923) writing on the pathogenic effects of *M. albicans* finds that subcutaneous inoculation in the guinea-pig produces only local infiltration and intraperitoneal inoculation is not followed by severe symptoms. Intravenous injection, however, is followed by death in few days with a generalised mycotic septicæmia. The monilia could be recovered from the liver, spleen and kidneys after death. In rabbits the results were similar and following intravenous inoculation the kidneys showed white mycotic nodule. Mice died with generalised moniliasis after intraperitoneal inoculation. The toxic principle is the living yeast as he found that the use of filtered cultures of these killed by heat did not result in death.

Hines (1924) found that *M. psilosis* recovered from cases of sprue killed guinea-pigs when introduced by the peritoneal route, whereas 'wild yeasts' from other sources were non-pathogenic.

Smith, Lawrence Weld (1924) has detailed extensive animal experiments. Five experiments by feeding monkeys are described. Four showed no symptoms except a temporary frothy diarrhœa which soon subsided. One showed a rise in temperature, frothy diarrhœa and later death from broncho-pneumonia. There were mild changes in the alimentary tract including several small superficial ulcers in one of which mycelia were demonstrated histologically. Monilia were recovered from the ileum and colon.

In a series of 24 guinea-pigs, half of which were fed with monilia and half of which received intraperitoneal injections, definite lesions of the gastrointestinal tract were found in two cases. Smith then fed his next series of animals on a diet deficient in vitamin C and then infected them with various species of monilia. Those fed with *M. albicans* showed only a transient gastrointestinal infection, whereas those infected with *M. psilosis* presented a more definite train of symptoms. Out of 12 guinea-pigs in the latter experiment ten died at periods averaging  $4\frac{1}{2}$  months. The animals lost weight, showed varying amounts of diarrhœa and after death showed lesions which were confined to the

gastro-intestinal tract particularly of the tongue, ileum and colon His conclusions are as follows —

‘Experiments support Ashford’s theory of the specific etiology of sprue as due to *M psilosis* by the production in guinea-pigs of a chronic moniliasis with gastro-intestinal lesions roughly comparable to those found in man

The development of the lesions, however, was rarely noted except in animals fed on a diet partially deficient in vitamins, especially the antiscorbutic vitamins This favours the view that a deficient diet, or diminished resistance such as is common following long residence in the tropics associated frequently with gastro-intestinal infections such as amoebic dysentery are contributory factors in its production’

The present writers do not place much importance on the recovery of monilia from the intestinal tract of experimental animals as they have found that in monkeys at any rate monilia indistinguishable from *M psilosis* are present in healthy animals Secondly, they have shown that severe intestinal lesions are found to result from a diet deficient in vitamin C quite apart from exposure to infection with monilia

The diarrhoea emaciation and death of the guinea-pigs described by Smith are symptoms common to scurvy, dysentery, chronic diarrhoea, and other similar conditions and cannot be accepted as an evidence of sprue This brings us back to the ever recurring difficulty in dealing with sprue, namely, that there is no criterion or diagnostic sign of this disease even in man much less in laboratory animals

Smith’s conclusions, though interesting and significant, cannot be held to prove the transmission of sprue to animals in view of the result recorded in this paper His experiments do show the importance of avitaminosis in the production of intestinal disease, results which we amply confirm

#### SUMMARY AND CONCLUSIONS

(1) When *M psilosis* (Ashford) and other species of monilia are injected into animals by the peritoneal route, a small proportion die of peritonitis with or without a generalised monilia septicæmia A larger number show a mild degree of plastic peritonitis and recover Many recover without any signs at all These conditions hold whether one or repeated doses of monilia are given

(2) No exaltation of virulence results from passage of *M psilosis* through a series of animals by intraperitoneal injection

(3) Feeding experiments have been negative when healthy animals were used The faeces of healthy animals frequently show the presence of monilia which are sometimes indistinguishable from *M psilosis* (Ashford)

(4) When monkeys are reduced to a scorbutic or subscorbutic condition by being fed on a diet deficient in vitamin C, a gastro-intestinal condition resembling dysentery, almost invariably results

(5) Sixteen such experiments on monkeys were performed Nine were fed on dilutions of sprue faeces or on culture of monilia, and of these all developed inflammatory changes in the gastro-intestinal tract, particularly in the large

As has been stated, Aschoff and his co-workers were the first to describe the reticulo-endothelial system as such, and to direct attention to its functional unity. They introduced the term 'histiocyte' to designate the mononuclear phagocytes of the connective tissues, and the term 'blood histiocyte' to indicate the mononuclear phagocytes of the circulating blood which they considered to be derived from the 'histiocytes' or R E cells of the tissues.

Within recent years this system has attracted considerable attention, and the literature on this subject is rapidly accumulating. It is evident that the vast majority of workers are agreed that the R E system must be regarded as a definite organ of the body, which, scattered though it undoubtedly is, functions as a unit, and has a definite rôle in both health and disease.

### III THE ANATOMICAL DISTRIBUTION OF THE R E SYSTEM

Aschoff (1924) has divided the R E system into two main groups, (a) the stationary group, and (b) the wandering group.

#### (a) *The stationary group*

Although differences of opinion exist as to whether certain cells belong to the R E system or not, most authors are agreed that the following should be included —(1) the *reticulum* cells of the splenic pulp, lymphatic tissue, and bone marrow, and (2) the *endothelial* cells of the sinuses of the liver, spleen, bone marrow, and lymph nodes. It is better for the present not to include those cells regarding the exact nature of which there is still doubt, but it is necessary to keep an open mind on this point. Piney (1926) states that only those cells which have a primary avidity for coloured or colloidal substances can be considered as belonging to the R E system.

*The spleen*—The structure and functions of this organ have always presented great difficulty, and it must be admitted that they are still imperfectly understood. The spleen in mammals is the organ which is richest in R E cells, and it is generally accepted that the most important of these are the reticulum cells of the splenic pulp, and the endothelial cells lining the sinuses. It is therefore necessary to refer to the most recent work on the finer structure of this organ.

Robinson (1926) has shown that in most instances the end arteries of the spleen terminate by emptying directly into the pulp spaces which are formed by joined R E cells. According to this authority 'the splenic pulp consists of a net-work of R E cells whose protoplasmic processes unite with one another to form a labyrinthine system of intercellular spaces through which the blood flows'. He considers that the structure and cellular content of the ellipsoids are the same as that of the pulp, but in more compact form. McNeal, Otani, and Patterson (1927) substantiate this view, and state that the circulation is essentially an open one, in that the pulp spaces constitute the connecting channels between the arterial capillaries and the venous sinuses, a mechanism which brings about intimate contact between the blood plasma and the cellular elements of the

with neutral red the dye granules are scattered diffusely through the protoplasm, but frequently the dye granules are scanty or may be entirely absent so that the cell may appear as an unstained hyaline cell

(2) The *lymphendotheliocyte* which is believed to be derived from the lymphatic reticulo-endothelial system When stained supra-vitally with neutral red, this cell is characterized by a focus of dye granules in the cytoplasm situated at one side of the nucleus in the shape of a 'rosette' or 'wreath'

(3) The *true monocytes* ('monocytes of Naegeli') which are, according to McJunkin, the most numerous of the mononuclear phagocytes in the peripheral blood and the only ones which give a positive peroxidase reaction with benzidine (*vide infra* Method C) McJunkin believes that these cells are derived from the bone marrow to a less extent from the splenic pulp

Aschoff (1924) quotes Kiyono as having by means of supra-vital staining with toluidine-blue shown that there are three types of mononuclear cells in the blood

(1) The blood histiocytes which arise from the reticulo-endothelial cells of the liver, spleen, and bone marrow

(2) The transitional cells of Ehrlich which arise in the bone marrow and apparently belong to the myeloid series (monocytes of Naegeli)

(3) The mononuclear cells of lymphoid origin (lymphocytes)

#### IV SYNONYMY OF THE VARIOUS TERMS APPLIED TO THE MONONUCLEAR CELLS OF THE PERIPHERAL BLOOD

In Table I an attempt has been made to co-relate in tabular form the various terms which have been applied to the mononuclear cells of the peripheral blood, in accordance with the reaction which they give when differentiated by the methods to be described below (*vide infra*, Methods)

This table is admittedly incomplete, and in certain instances there is some degree of overlapping, but it is hoped that it may enable the types of cells which have been recently described to be better understood The terms which have been applied to the mononuclear cells of the peripheral blood are numerous and confusing, and it is regrettable that the same name has, in at least one instance, been applied to two totally different types of cells The more recent contributions to the literature on this subject show that the views of the various authors are coming more and more into line, and as has already been stated the differences of opinion are now more apparent than real, and due in a large measure to the unfortunate terminology

Practically all writers are agreed as to the functional and morphological identity of the 'lymphocytes' with the cells of lymphoid tissue These cells do not react to carbon in suspension, neutral red in supra-vital preparations, or peroxidase staining with benzidine

Mention may be made in passing of the polymorphonuclear neutrophils, regarding the nature and origin of which there is no disagreement These cells

follicles, and a similar contact between the formed elements of the blood and the R E cells of the marginal zone and the pulp cord Tait (1927) expresses a similar view and says, 'The ellipsoidal mode of termination of the arteries is a fundamental feature of splenic architecture, and the sessile phagocytic cells of the ellipsoids capture foreign particles introduced into the blood stream and probably also ingest effete corpuscular elements of the blood'

*The liver*—The stellate or Kupffer's cells form the most important part of the R E system in the liver It is not yet definitely settled whether these cells are true endothelial cells, or modified reticular elements (Sacks, 1926) They form the incomplete lining of the sinusoids of the liver and lie in direct contact with the blood on one hand and the liver cells on the other McNee (1924) in describing his view on the structure of the hepatic lobule says, 'between the tubular glands run the wide portal vascular capillaries passing from the portal tract to join the branches of the hepatic vein in the centre of the lobule Along the walls of these capillaries lie a number of large endothelial cells (Kupffer's cells) which form an important part of Aschoff's R E system'

*Bone marrow*—Schafer (1920) states that there is no interstitial circulation of blood in the bone marrow such as is found in the spleen He quotes Tait and McNaughton as having shown that the capillaries of the bone marrow are similar in their behaviour to inorganic particles (e.g., India ink) to the blood channels of the liver and spleen, the endothelial cells tending to engulf such particles Doan (1922) has, however, shown that the real functioning bed of the marrow is formed by the very extensive distribution of the large lumened, thin walled, venous sinusoids, and has in addition demonstrated the presence, hitherto unsuspected, of an extensive inter-sinusoidal capillary plexus, which is probably in a state of collapse under normal conditions This writer is of opinion that the endothelium of the sinusoids forms a continuous lining throughout the vascular ramifications in the bone marrow, being very extensively distributed through the medium of the widespread capillary plexus

Peabody (1926) agrees with Doan as to the presence of these inter-sinusoidal capillaries and believes that they are compressed sinuses which, after stimulation, open up and show definite endothelial proliferation Muller (1926, 1927) appears to agree with these findings and has succeeded in producing varying degrees of reaction on the part of the capillary endothelium of the bone marrow following injections of collargol It is considered that these proliferating endothelial cells can be included in the R E system

*Lymph nodes*—The lymph nodes are small bean-shaped bodies which are scattered along the lymphatic vessels Their sinuses are lined by an imperfect layer of flattened plate-like cells which are a continuation of the endothelium of the adjoining lymphatic vessels The passage of lymph through a node is retarded by the reticulum within the sinuses thus favouring a process of phagocytosis Not only the fixed cells of the reticulum and endothelium participate in this process, but also the free cells within the lymph sinuses

may ingest small particles of carbon in suspension, and are invariably positive to the peroxylase reaction with benzidine. In supra-vital preparations the polymorphs are easily distinguished by their finely granular and faintly staining cytoplasm, and by their strikingly active motility.

It is with regard to the mononuclear phagocytes that most difficulty is experienced.

Sabin and Doan (1926) describe a large mononuclear cell in supra-vital preparations which does not react, or only to a slight extent, to neutral red. This type of cell is included by them in their 'endothelial phagocytes' (a term used synonymously with 'clasmatocyte'). This cell, which reacts partially or not at all to neutral red, is considered to be identical with that described by McJunkin as the 'hemendotheliocyte'. It may therefore be inferred that the hemendotheliocytes of McJunkin correspond to the clasmatocytes *pro parte* of Sabin and her co-workers. Since both these authorities are agreed as to the endothelial origin of these cells it seems probable that they correspond to the 'endothelial' or 'desquamated endothelial' cells of some of the older authors, and to the 'blood histiocytes' *pro parte* of Aschoff. These cells give a positive reaction to peroxylase staining with benzidine only when they have ingested positive reacting material.

The 'lymphendotheliocytes' of McJunkin, and the 'monocytes' of Sabin and her co-workers are evidently identical, since each of these authorities has described under the terms stated a type of cell which, when supra-vitally stained, takes up neutral red in the form of a 'rosette'. Both schools are agreed as to the nature of the tissue from which this 'rosette' cell is derived. McJunkin is of opinion that the term 'monocyte' should be reserved for those cells which give a positive peroxylase reaction with benzidine, and since he believes that the 'rosette' type of cell is non-peroxylase reacting he claims that the term 'monocyte' is erroneously used by Sabin and her co-workers. Doan and Sabin (1926) hold a different view with regard to the cells which give a positive peroxylase reaction, and state that with the Sato and Sekiya technique (*vide infra* Method C) the majority of the monocytes of the human blood are positive but both positive and negative reactions are found. McJunkin considers that his 'lymphendotheliocyte' corresponds exactly to the 'large mononuclears' of the older authors. The 'rosette' type of cell (lymphendotheliocyte or monocyte) is considered to correspond to the 'blood histiocytes' *pro parte* of Aschoff.

The 'true monocytes' of McJunkin are believed by him to be the only peroxylase reacting mononuclear cells of the peripheral blood, with the exception of a few which may give a positive reaction in virtue of having ingested positive material. McJunkin considers that his 'monocytes' correspond exactly to the 'monocytes of Naegeli,' and to the 'transitionals of Ehrlich,' and in this he is in agreement with Aschoff and Kiyono (Aschoff, 1924). The views of Sabin and her co-workers on the peroxylase reacting mononuclear cells have already been given.

(b) *The wandering group*

This group includes the R E cells of the circulating blood, the free cells of the connective tissues, and the large free cells in the sinuses of the lymph nodes and spleen

*The large mononuclear cells of the circulating blood*

The theories as to the nature and origin of the large mononuclear cells of the blood are too numerous to discuss in detail, and mention will only be made of the more recent work on this subject

The observations of Aschoff and Kiyono (Aschoff, 1924), of McJunkin (1925, 1926), and of Sabin, Doan, Cunningham, and others (1923, 1924, 1925, 1926), have thrown fresh light on the nature of these cells, and although the findings of these writers cannot be said to be in complete agreement, it is evident that all are in accordance with the conception of the R E system as a functioning unit

Sabin and her co-workers divide the mononuclear cells of the peripheral blood into three groups, lymphocytes, monocytes, and clasmatocytes, and since the two latter groups react to neutral red in supra-vital preparations, they may be considered as belonging to the R E system. According to the Sabin school the monocytes arise from primitive reticular elements which are abundant in the spleen and bone marrow, while the clasmatocytes arise from endothelium. The variation in size of these clasmatocytes suggests that they arise from different endothelia, but these workers do not attempt to differentiate them on a basis of origin. Many so-called lymphocytes are really clasmatocytes, and their differentiation can be made by means of the supra-vital technique.

McJunkin classifies the mononuclear cells of the blood according to their source of origin, and believes that there are four types in the peripheral blood, (1) hemendotheliocytes, arising from blood vascular endothelium, (2) lymphendotheliocytes, arising from lymphatic endothelium, (3) monocytes, arising from the splenic pulp and bone marrow, and (4) lymphocytes, arising from lymphoid tissue.

Aschoff and Kiyono divide the mononuclear cells of the blood into three groups, (1) blood histiocytes, which arise from the R E cells of the tissues, (2) transitional cells of Ehrlich which have their origin in the bone marrow, and (3) lymphocytes, which arise from lymphoid tissue. Only the blood histiocytes can be considered as belonging to the R E system.

This aspect of the R E system will be dealt with more fully in a later paper.

#### IV FUNCTIONS OF THE R. E. SYSTEM

The cells of the R E system have the power of taking up certain coloured substances, colloidal suspensions, or insoluble particulate matter, by a process of phagocytosis. In animals after suitable injections of India ink, or other insoluble





particles in suspension granules of the pigment can be found in the cells of the R E system, which in most instances can be differentiated by such methods

Gye and Purdy (1922, 1924) found that after several injections into rabbits, of colloidal silicic acid at intervals of a few days, there was enlargement of the fixed histiocytic cells of the liver, spleen, and bone marrow, and that these cells were subsequently liberated into the circulation Simpson (1922), using several different stimulating agents to produce macrophages in the circulating blood, found that the only factor common to these agents was that each contained some substance in the colloidal state She noticed that in animals which had received repeated injections, macrophages were given off in 'showers' after each succeeding injection Macrophages thus produced were unevenly distributed in the circulation, being most abundant in the venous blood of the liver and spleen, and either absent, or present only in small numbers, in the peripheral blood

Within recent years it has been found possible by means of massive injections of India ink, colloidal suspensions, etc., to throw the R E cells out of action The effect thus produced is now commonly known as 'blockage' of the R E system

The following experimental methods have been employed in animals to eliminate the R E system — 'blockage,' splenectomy, and hepatectomy The method most usually employed is 'blockage,' or 'blockage' combined with splenectomy Mann and others (1926) have succeeded in performing hepatectomy in dogs and in keeping the animals alive for some time afterwards, and have even successfully performed splenectomy and hepatectomy in the same animal

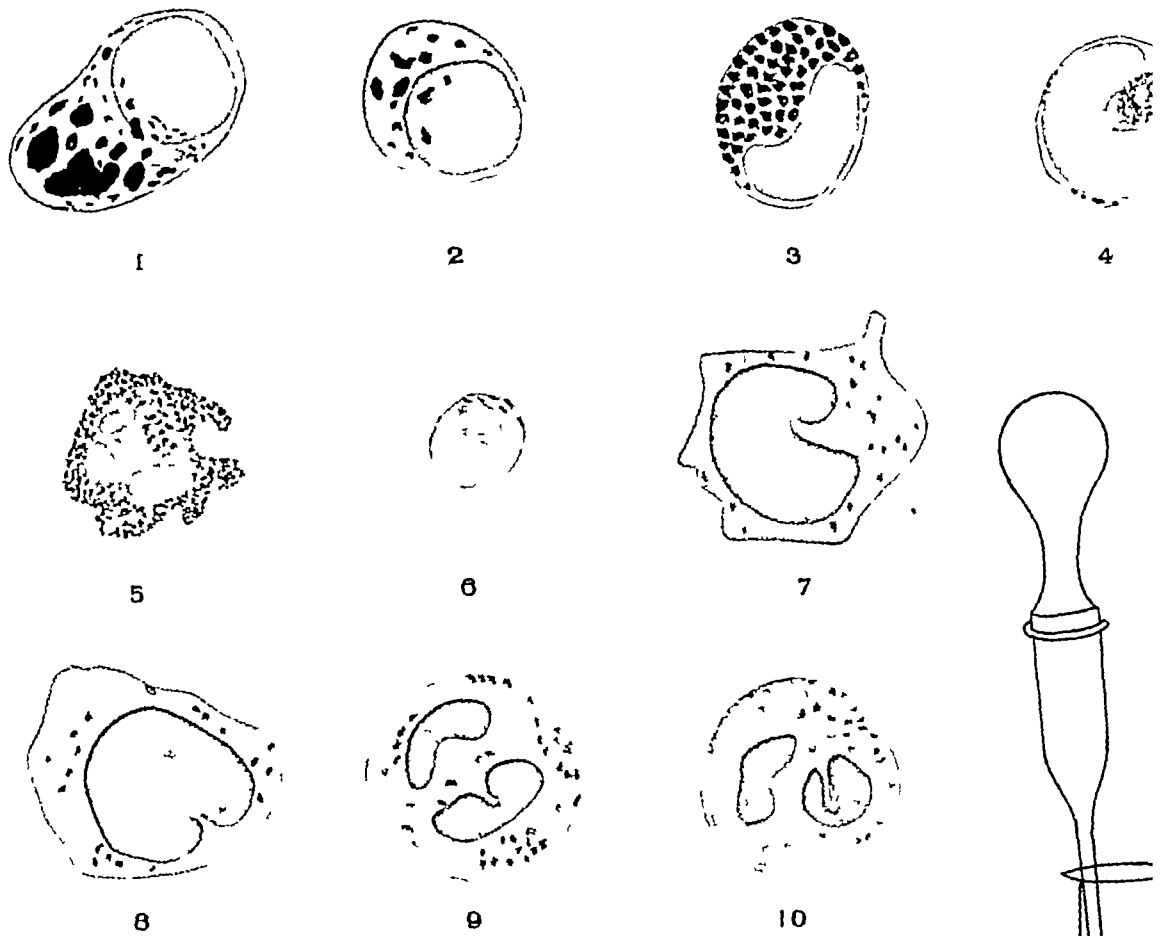
Much experimental work has been done to determine the part played by the R E system in certain physiological processes, and as the result of this work considerable evidence has accumulated to show that this system is closely related to the following —

- (1) Regulation of certain formed elements of the blood
  - (a) mononuclear phagocytes,
  - (b) red blood cells,
  - (c) blood platelets
- (2) Blood destruction and bilirubin formation
- (3) Chemotherapeutic action of drugs
- (4) Immunity and anaphylaxis
- (5) Certain metabolic activities

It has been considered advisable to give a very brief account of the R E system in general, before taking up the study of any special function

It will be seen from the above review that the R E system tends to be thrown out of action following injections of certain colloidal suspensions, and particulate matter Some authorities believe that small doses of such particulate matter act on the R E system in the same manner as large doses but to a less extent, while others are of opinion that small doses may exert a stimulating effect on this system It is thought that the enormous amount of pigment,

# PLATE LXXXVII



## EXPLANATION OF PLATE

- Fig 1—'Hemendotheliocyte' (McJunkin) supra-vitally stained with neutral red after phagocytosis of carbon in vitro (Method A)  
 „ 2—'Hemendotheliocyte' (McJunkin) prepared as described in Method A  
 „ 3—'Clasmatocyte' (Sabin, etc), supra-vitally stained with neutral red and Janus green  
 „ 4—'Monocyte' (Sabin, etc), or 'lymphendotheliocyte' (McJunkin) stained as in 3  
 „ 5—Polymorphonuclear leucocyte stained as in 3  
 „ 6—Small lymphocyte stained as in 3  
 Figs 7 and 8—'Monocyte' (McJunkin) stained as described in Method C  
 „ 9 and 10—Polymorphonuclear leucocyte stained by Method C  
 Fig 11—Special tube as used in Methods A and C  
 (a) White blood corpuscle layer  
 (b) Red blood cells

(Diagrammatic Not drawn to scale)

parasites, and cellular debris, which is present in the circulation during certain stages of the malarial fevers, may act on the R E system in a manner similar to the substances which have been used experimentally in animals in producing 'blockage,' e.g., India ink, collargol, etc. It is impossible to estimate the 'dose' of the pigment, cellular debris, etc., in the blood of malaria patients, and therefore impossible to forecast the effect (stimulating or depressing) on the R E system which is likely to result. In order to determine to what extent the R E system is involved in malaria, it is proposed in future studies to investigate in detail the relationship between malarial infection and the known physiological functions of the R E system, and as each is taken up, a more detailed account of our present knowledge of this subject will be given.

The effects of malaria may be reflected in the R E system by —

- (1) Changes in certain of the formed elements of the blood, e.g., mononuclear phagocytes, blood platelets, etc
- (2) Changes in the R E cells of the tissues and organs
- (3) Changes in the pigments formed in the body such as bilirubin, urobilin, etc
- (4) Alterations in certain immunological processes and anaphylaxis
- (5) Alterations in certain metabolic activities

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*Investigation of the nature of the large mononuclear phagocytes in the peripheral blood in malaria*

In view of these recent observations it was considered that some fresh light might be thrown on the nature and origin of the mononuclear phagocytes in malaria using as means to this end the method of phagocytosis of carbon *in vitro*, of supra-vital staining with neutral red and Janus green, and of peroxydase staining with benzidine

## V METHODS

*Method A*—This method is a modification of that described by McJunkin (1925), and was used to differentiate and study those mononuclear cells in the peripheral blood of malarial patients which showed a marked tendency to phagocytose carbon particles in suspension

To a specially constructed small glass tube<sup>1</sup> containing 1 c c of a suspension of India ink in sodium citrate solution<sup>2</sup>, 1½ c cs freshly drawn blood are added, and the tube placed in an incubator at 37°C for 10—15 minutes, removed and centrifuged at a comparatively low speed for 10 minutes and at a high speed for 5 minutes. After carefully pipetting off the supernatant fluid the tube is replaced in the incubator for a further 10—15 minutes, after which smears are made on cover glasses<sup>3</sup> from the white blood corpuscle layer, dried and counter-stained

A quick and convenient method of counterstaining these smears, whereby the cell nuclei are clearly defined and the carbon particles are easily distinguished, is furnished by the use of an aqueous solution of safranin (Plate LXXXVII, fig 2) employed in the same manner as that described under Method C for counterstaining peroxydase smears

*Method B*—The technique of this method is the same as that described by Sabin (1923). By this means an attempt was made to determine the proportion of mononuclear phagocytes in malarial blood which reacted to supra-vital staining, and the type of reaction produced

It is absolutely essential to have the slides and cover slips free from the smallest trace of grease and dust. To ensure this they are kept for 3 or 4 days in concentrated sulphuric acid to which a few crystals of potassium bichromate have been added. They are then placed in running water for several hours taking care to see that the water gets between the slides, as any trace of acid

<sup>1</sup> Small glass tubes were prepared with an elongated constriction near the closed end in such a manner that after centrifuging a measured quantity of diluted blood the leucocyte layer corresponded to the middle of the constriction. The deep leucocyte layer thus presented was much more easily pipetted off than in the case of an ordinary small-bore test tube where the leucocyte layer, which is located in the full bore of the tube, was very shallow. This device (Plate LXXXVII fig 11) enabled several smears to be prepared from a small volume of blood

<sup>2</sup> This is a 3·8 per cent sodium citrate solution to which Higgin's insoluble India ink had previously been added in the proportion of two drops of ink to 5 c c citrate solution

<sup>3</sup> Long cover glasses 2 inches by ½ inches were found to be convenient for this purpose

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would be injurious to the living blood cells. The next step is to rinse them 2 or 3 times in distilled water in which they are left overnight, again washed, and finally transferred to 80 per cent alcohol in which they are stored. Before use the slides are removed from the alcohol with forceps (they must not be touched by the fingers until the film has been made), dried with a clean silk handkerchief, and highly polished with jeweller's rouge. The slides are then ready to have a dye film made on them.

In the experiments to be described a mixture of neutral red and Janus green was used. The former is said to be the least toxic of the vital dyes, and the latter greatly facilitates the recognition of the lymphocytes by staining their mitochondria a bright bluish green. It was found convenient to keep two stock bottles of saturated solutions of neutral red and Janus green in absolute alcohol and to make up the diluted stain as required. The most satisfactory mixture for the examination of ordinary peripheral blood preparations was found to be 20—30 drops of the stock solution of neutral red and 8—10 drops of the stock solution of Janus green in 10 c.c. of absolute alcohol. Stronger mixtures are necessary for the examination of preparations of a more cellular nature such as spleen puncture fluids, and samples from the leucocyte layer referred to in Method A.

Thin even films of the supra-vital dyes are prepared by flooding the cleaned and flamed slides with the diluted mixture, quickly draining off the excess of stain, and standing the slides vertically to dry. No difficulty was experienced in ordinary weather in obtaining thin even films in this manner, but in the rainy season better results were obtained if the slides were very gently warmed after draining off the excess of stain. The diluted mixture may be used repeatedly so long as it is kept free from grease and dust. The prepared slides must be stored in dust-proof boxes.

To make supra-vital preparations of blood from the peripheral circulation, a small drop of blood from a finger prick is taken on a thoroughly cleansed cover slip, which is then inverted and placed on a prepared slide, when the blood spreads out into a thin even film. The cover slip should be ringed immediately with paraffin of a melting point above  $37^{\circ}\text{C}$ , i.e., above the temperature of the warm box. Such a preparation must be kept at  $37^{\circ}\text{C}$  in a warm box or on a warm stage and the count must be completed within half an hour of the time of preparation.

*Method C*—In order to determine the proportion of peroxidase reacting cells in the peripheral blood of malarial patients, the method of peroxidase staining with benzidine as described by Sato and Sekiya (1926), but slightly modified, was employed.

Leucocytes for examination by this method were obtained in a manner similar to that employed in Method A, the only difference being that the freshly drawn blood was added to sodium citrate solution without India ink.

The following materials are required —

Solution (a) 5 per cent aqueous solution copper sulphate

# STUDIES ON THE RETICULO-ENDOTHELIAL SYSTEM WITH SPECIAL REFERENCE TO MALARIA

## Part II.

### THE LARGE MONONUCLEAR CELLS IN THE PERIPHERAL BLOOD IN MALARIA

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#### I INTRODUCTION

THE leucocytes, and particularly the large mononuclears, in the peripheral blood in malaria, have always attracted considerable attention more especially since the introduction of the Romanowsky method of staining and its various modifications. It is impossible here to review all the literature which has accumulated on this subject, but it may be stated that however widely the innumerable contributors may differ in the details of their observations, two findings are outstanding for their constancy, namely, (1) the presence of a relative increase in the large mononuclear cells, and (2) the presence of a leucopenia

Mononucleosis is most marked in the apyrexial intervals between malarial paroxysms, and least marked during the febrile periods. Thomson (1911) and Seyfarth (1926) have shown that the percentage of total mononuclears in malaria is the exact inverse of the temperature curve. In former times the presence of an increase in the percentage of the large mononuclears was regarded as a point of primary diagnostic significance in malaria, but more recent researches into other tropical blood conditions have proved that this factor must now be considered as being of only secondary diagnostic value. Acton and Knowles (1912) are of opinion that a high relative percentage of mononuclears is suggestive of protozoal infection, and a fluctuating leucocyte count with a high mononuclear percentage at the leucopenic stage is very suggestive of the presence of malarial parasites in the body. In a recent paper Dutton (1928) goes so far as

( 1107 )



(b) Benzidine solution This is the filtrate obtained after rubbing up 0.2 grammes of benzidine with a few drops of water in a mortar, adding 200 c.c. distilled water, and filtering

(c) 1 per cent solution hydrogen peroxide

(d) 0.05 per cent aqueous solution safranin

Before commencing add 1 drop of solution (c) to 5 c.c. of solution (b) and shake (It was found more satisfactory to add the hydrogen peroxide solution to the benzidine solution immediately before use)

Cover a fresh dry blood smear prepared as described with solution (a), drain off the excess quickly, and cover with the mixture of solutions (b) and (c) for 2 minutes, and wash in distilled water. Place 1 drop of solution (d) on a clean slide, and invert upon it the peroxydase stained smear, ring with vaseline and examine under 1/12 inch oil immersion. The peroxydase granules are stained a bright bluish green.

Sacks (1926) is of opinion that although lymphocytes and monocytes can be differentiated by means of ordinary hæmatological stains, this can be accomplished with greater accuracy by means of the supra-vital technique. It must be remembered that it is possible to count only a comparatively small number of cells (100—150) in the half hour following the preparation of a supra-vital stained blood film. Stephens and others (1921) have shown that in making dried films the value of a differential count is impaired owing to the uneven distribution of the cells, and owing to the retention of a certain number of cells by the spreader. These disadvantages are obviated to a considerable extent by the methods used in making supra-vital preparations.

In the two series of cases referred to below, in addition to the methods already mentioned, total white cell and parasite counts were made on each case by the method evolved by Sinton (1924). In a certain number the hæmoglobin index has also been recorded.

## VI MATERIAL

Two short series of cases were examined, and the results are shown in Tables II and III.

In the first series (Table II), the patients were all young European soldiers suffering from chronic benign tertian malaria, which in each case had shown a tendency to relapse in spite of repeated courses of treatment. The patients had consequently been sent to the Malaria Treatment Centre, Kasauli, for special treatment. In all cases the blood examinations recorded below were carried out within a few days of the last rigor, and after the parasites had disappeared from the peripheral blood. The majority were undergoing quinine treatment, but a few were being treated with plasmoquine either alone or in combination with quinine.

In the second series (Table III), the patients were Indian sepoy (mostly Gurkhas), and included cases of benign tertian, quartan, and malignant tertian malaria. The majority of these cases were examined in the more acute stages of

to state that a mononucleosis of 6 per cent or over, unless in certain specified conditions, is proof of protozoal infection

With regard to the influence of quinine on these cells Gruner (1913) cites quinine as a causative factor in the production of monocytosis, and in large doses, of leucopenia Zweig (1916) states that the changes in the number of the mononuclear cells in malaria occur independently of quinine treatment, while Fergusson and Thomas (1924) consider that the therapeutic action of quinine depends on its ability to stimulate the production of leucocytes

Leucopenia may still be regarded as a sign which is suggestive of malarial infection, though it must be remembered that it is by no means constant and that it does occur in other diseases, e.g., kala-azar, dengue, relapsing fever, etc. At the commencement of the malarial paroxysm there is frequently a leucocytosis [Stephens and Christophers (1908), Castellani and Chalmers (1919), Seyfarth (1926)] Thomson (1911, 1912) has shown that there may be a post-malarial leucocytosis occurring daily in patients who are undergoing quinine treatment, and has brought experimental evidence to support his belief that a leucocytosis is probably caused by the presence of a small number of parasites, and a leucopenia by a large number, hence the variations in leucocyte counts recorded several weeks after the disappearance of parasites from the peripheral blood Acton and Knowles (1912) consider that a leucopenia of 2,000—7,000, or a leucocytosis of 16,000 or over in the absence of other obvious cause, is very suggestive of malaria, while Bouchard (1924) mentions both mononucleosis and neutropenia as signs of latent malaria

## II OLDER WORK ON THE LARGE MONONUCLEARS IN MALARIA

Although, as has been stated, most writers are agreed on the constancy of a relative increase of the large mononuclear cells in malaria, there is considerable diversity of opinion with regard to the actual percentage increase which is to be taken as of diagnostic significance. This is easily understood when it is remembered that there is no unanimity of opinion as to the exact type of cell which should be recorded as a 'mononuclear'. It must also be kept in mind that recorded differential counts are of comparatively little value unless the worker and his personal equation are known. The majority consider that it is impossible to distinguish with certainty more than two groups of mononuclear cells (a) 'lymphocytes,' and (b) 'large mononuclears and transitionals,' while others have, in addition to these, described such cells as 'macrophages,' and 'desquamated endothelial cells'. It is convenient at this point to refer to the definitions of the different types of mononuclear cells which have from time to time received most attention

Stott (1915), in a consideration of the value of the large mononuclear count in malaria, states that it is simple and safe as a general rule to follow Roger's axiom and classify all mononuclear cells larger than the average polymorph as 'large mononuclear,' and all such cells of a lesser dimension as 'small mononuclear'. He considers that the large white cells with kidney-shaped nuclei ('transitionals' of Da Costa and Daniels) to be typical 'large mononuclear'. He counts along with the 'large mononuclears' those cells of a similar size,

the disease, but not until the end of the paroxysm when it was expected that with the falling temperature, the mononuclears would be on the increase [*vide* Thomson (1911) above] It is highly probable that the majority of these cases were suffering from fresh infections of malaria, though it is very doubtful whether any of them were first infections In a number of instances the Medical History Sheets showed entries for malaria, while in those where this information was not available, the majority of the patients gave a history of previous similar attacks, but in the case of Indian troops such a history is of little value

## VII RESULTS

The results are shown in Tables II and III The blood counts have been tabulated according to the clinical condition of the liver and spleen, since these are the only hæmolytopoietic organs which lend themselves to bed-side investigation

In the tables each section has been divided into four columns marked C, N R (D), N R (R), and B Under column C are grouped those cells which showed a marked tendency to phagocytose carbon *in vitro* (hemendotheliocytes of McJunkin, and clasmatocytes *pro parte* of Sabin and her co-workers) In the column headed N R (D) are shown those cells which, when treated with neutral red in supra-vital preparations, showed granules scattered throughout the protoplasm without definite pattern (hemendotheliocytes *pro parte* of McJunkin, and clasmatocytes of the Sabin school) Those cells which, in supra-vital preparations, showed neutral red granules in the form of a 'rosette' (monocytes of Sabin and her co-workers, and lymphendotheliocytes of McJunkin) are shown in the column marked N R (R) In column B are grouped those cells which gave a positive peroxydase reaction with benzidine (monocytes of McJunkin, and monocytes *pro parte* of the Sabin school)

All authorities are agreed that those mononuclear cells of the blood which have a marked affinity for vital dyes, and for particulate matter in suspension belong to the R E system In Tables II and III the R E cells are represented by the sum of the cells in columns C, N R (D), and N R (R)

The bloods of a number of normal persons were examined by the methods given above, and it was found that the R E cells were present in the following proportions —

Hemendotheliocytes (carbon positive) 0.0 per cent

Clasmatocytes [N R (D)] 0.5 to 1.5 per cent

Monocytes and Lymphendotheliocytes [N R (R)] 2 to 3.5 per cent

Monocytes of McJunkin (benzidine positive) 3 to 4 per cent

Reference to Tables II and III will show that in both acute and chronic malaria there is a marked increase above normal in the R E cells, and that generally speaking this increase is not confined to any particular type of cell, but affects all It is possible that the products of activity of the malarial parasite stimulate the R E system in a manner comparable to the effects produced by intravenous injections of such substances as India ink

occasionally known as large lymphocytes, which contain a large, faintly staining, irregular, oval or square-shaped nucleus, and also the so-called 'endothelial cells' which are as large as any of the above, but having a small, distinct, round, or ovoid and well stained nucleus. These three groups have two factors in common (1) size, and (2) a plentiful supply of non-granular protoplasm. This latter property has given rise to another name, i.e., 'hyaline cell'.

Armstrong (1916), in a paper in which he supports the views of earlier authors that the increase in the 'large mononuclears' is so constant as to be trustworthy evidence of malaria, defines the 'large mononuclears' when stained with the Romanowsky stains as follows — 'it must be at least as large as the largest polymorph present the nucleus which is woolly in appearance, and either ovoid or reniform, but very rarely squarish and never round, must show less depth of staining than any other white cell in the film (unless the mast cell when its nucleus fails to stain), the colour with Leishman's stain being a cherry violet as opposed to the bluish purple of the large lymphocyte'. Later the same writer mentions two rare cells (the 'transitional' and the 'endothelial') as being numbered with the mononuclears.

James (1922) has described the 'macrophage' as a cell 15  $\mu$  or more in diameter, bluntly oval in shape, with a kidney-shaped nucleus which is eccentric or, a more or less circular cell in which the nucleus is rounder and more centrally placed in the hyaline protoplasm. The same author has described the 'desquamated endothelial cell' as being larger than the former, and more irregular in shape, with hyaline protoplasm which is often vacuolated. This cell is not seen in normal blood. In both these types of cell malaria pigment, ingested red blood cells, and parasites may be found.

Hamner (1928) in an address on the interpretation of total and differential white cell counts writes as follows — 'That the majority of mononuclear cells are lymphocytes seems certain owing to their morphologic identity with the cells of lymphoid tissue, and the presence of some of these cells in the large lymph vessels. "Transitional leucocyte" is a term commonly given to leucocytes that have a nucleus more or less horse-shoe, or saddle-back in shape. The views as to the origin and identification of this cell are almost as numerous as the publications themselves. Most of the recent workers are agreed that this cell has granules when treated with the polychrome stain, that it has neutrophile granules when treated with Ehrlich's triacid stain, and that it gives the iodophenol reaction. Another common term used in blood work is "large mononuclear leucocyte". As in the case of the transitional this term, in regard to the use of which there is little uniformity, is not a histologic one, and gives no indication as to the origin of the cell. It differs from the transitional especially in not having a horse-shoe-shaped nucleus. There is not proof at hand that most of the cells classified under these two terms (mononuclear and transitional) arise from the endothelium of certain blood and lymph vessels. They are frequently mistaken for the large lymphocytes, but have more protoplasm, and a nucleus which stains rather faintly. Their differentiation is, however, very difficult'.

It is evident from a perusal of the literature on the leucocytes in malaria that, apart from the rather vague use of such terms as 'large mononuclear,' 'transitional,' 'macrophage,' and 'endothelial,' there is no clear conception of the nature and origin of the mononuclear cells which practically all writers are agreed are (at least relatively if not absolutely) increased in malaria. The vast majority of the investigations made hitherto on these cells have been done by the use of the Romanowsky stains and it is felt that any further progress must take place along fresh lines.

During the past few years several attempts have been made to investigate more fully the mononuclear and other cells of the blood in certain diseases of



the hæmolytic and hæmopoietic organs. The introduction of newer methods has facilitated not only the study of the tissue cells, and the cells of the vascular organs, such as the spleen, liver, bone marrow, etc., but also the cells of the peripheral blood. So far as is known no researches along these lines have been recorded in malaria.

### III RECENT WORK ON THE MONONUCLEAR PHAGOCYTES

It cannot be claimed that the results of the more recent investigations into the nature and origin of the mononuclear phagocytes of the peripheral blood are all in complete harmony, but a close examination shows that the discrepancies are more apparent than real.

Sabin (1923) applied a method previously used by her in the study of chick embryo cells (Sabin, 1921) to the study of living human blood cells. This method consists in the examination of fresh blood stained supra-vitally with neutral red, or neutral and Janus green.

Later Sabin and Doan (1926) using this technique, described two strains or types of mononuclear phagocytes in the blood, (a) a type of cell which takes up large quantities of insoluble dyes and segregates them in large masses, distributed in the protoplasm without definite pattern. This type of cell they consider to be derived from endothelium, and to it they have applied the name 'clasmatocyte,' a term originally introduced by Ranvier about fifty years ago. (b) a type of cell which takes up insoluble dyes in a different manner, namely, in the form of a rosette of small bodies surrounding the centrosphere, and staining constantly with neutral red. They have applied the term 'monocyte' to this cell, and in it phagocytosed material is found peripherally. This type of cell is considered to be derived more directly from the mesenchyme (reticular tissue). Both the 'clasmatocyte' and the 'monocyte' are strongly phagocytic.

Previously Sabin, Doan, and Cunningham (1925) had described two similar types of cells in peritoneal exudates, and this was confirmed by McJunkin (1925), who described in addition a third type of cell, namely, a hyaline type of cell in peritoneal exudates which readily phagocytosed carbon particles in suspension. McJunkin further noticed that this 'hyaline' type of cell reacted to neutral red and carbon in much the same manner as Kupffer's cells of the liver, and considered that these 'hyaline' cells which readily ingested carbon particles were derived from hemovascular endothelium of the type lining the sinusoids of the liver.

McJunkin (1926) later described three types of mononuclear phagocytes in the peripheral blood.

(1) The *hemendotheliocyte* which is the same type of cell as the hyaline cell referred to above. As the name indicates this cell is considered to be derived from the blood vascular endothelium. It does not appear in the peripheral blood under normal conditions, but it may appear in certain pathological conditions, and may be produced experimentally in the peripheral blood of rabbits following intravenous injections of large doses of India ink. When stained supra-vitally



intestine Clinical signs of dysentery were not seen in all these animals, not even in some of those which were found to show extensive changes in the large bowel after death

(6) Four control monkeys on normal diet remained healthy Three control monkeys fed on a scorbutic diet without any infective feeds of sprue materials died with inflammatory changes in the large intestine precisely similar to those found in the sprue-infected animals

(7) Monkeys kept on a diet deficient in vitamin C almost invariably develop inflammatory changes in the large intestine (apparently of a non-specific nature), whether they are fed with sprue material or not

(8) It is interesting to note that extensive changes in the large bowel of a dysentery-like nature are not necessarily revealed during life by clinical signs of dysentery or even of diarrhoea

(9) The final conclusion of this paper is that sprue has not been produced in monkeys or other laboratory animals

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#### KEY TO PLATE X

- Fig 1 Kidney of rabbit showing minute necrotic foci, the result of an intravenous injection of toxic monilia (life size)
- „ 2 Section of such a kidney showing the distribution of necrotic foci resulting from growth of monilia (20 approximate)
- „ 3 The same under higher power Leitz No 3 lens
- Figs 4, 5 and 6 Monilia growing in a necrotic focus
- (The exact magnification cannot be given owing to variations in the camera extension )

## VIII DISCUSSION OF RESULTS

It is fully realised that the number of cases examined is too small to warrant the drawing of any definite conclusions, but an examination of Tables II and III will show that there are certain interesting deviations from normal which deserve discussion, and which indicate that the subject is worthy of further investigation along similar lines

In approximately 70 per cent of all the cases examined a small number of cells (usually less than 1 per cent) corresponding to McJunkin's hemendotheliocytes was present. In both acute and chronic malaria the average number of these cells was greater in those cases in which there was enlargement of the liver, than in those where this organ was not palpable. It is interesting to note that cells of this type can be produced experimentally in the peripheral blood of rabbits following intravenous injections of large doses of India ink (McJunkin, 1926), and of certain substances in the colloidal state (Simpson, 1922)

In nearly every case there was an increase in the N R (D) cells (clasmato-cytes), the average number of these cells being greater in acute (4.3 per cent), than in the chronic cases (2.4 per cent). The increase in the number of these cells does not appear to bear any marked relationship to the clinical condition of the liver or spleen, though in the acute cases (Table III) the average number was slightly higher in those cases in which the spleen was enlarged.

The N R (R) cells (lymphendotheliocytes of McJunkin, or monocytes of Sabin) showed a practically constant increase, the average number in acute and chronic cases being almost identical (7.5 and 7.7 per cent respectively). In this instance also, the average was slightly higher in those cases in which the spleen was enlarged.

It will be noticed that in Table II those cases in which the liver or spleen or both were enlarged, the blood picture, as determined by the methods given above, approximated to that seen in the cases in Table III, whereas in those in which these organs were not palpably enlarged there was a striking disproportion between the number of clasmato-cytes and the number of monocytes, the latter being by far the more numerous (1.8 per cent and 7.6 per cent respectively). In the acute cases (Table III), and more especially those in which neither the liver nor spleen was palpable, the clasmato-cytes and monocytes were more nearly equal (4.5 and 6.25 per cent respectively).

With regard to the cells which gave a positive peroxydase reaction the most noticeable feature was that in both acute and chronic cases they varied within wide limits. According to the Sabin school, the vast majority of their monocytes (R E cells) are peroxydase reacting, while their clasmato-cytes are peroxydase reacting only when they have ingested material which is peroxydase reacting, e.g., fragments of blood cells, etc. McJunkin, on the other hand, states that the majority of the large mononuclear cells of the blood are peroxydase reacting, but he believes that his lymphendotheliocytes (which correspond to the monocytes of Sabin, etc.) are non-peroxydase reacting.



FIG 1



FIG 2



FIG 3

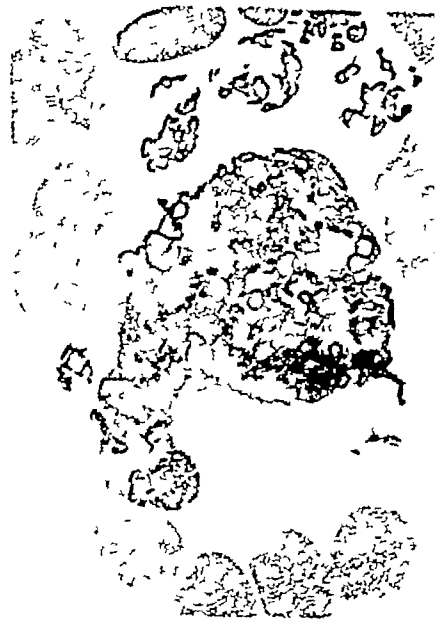


FIG 4

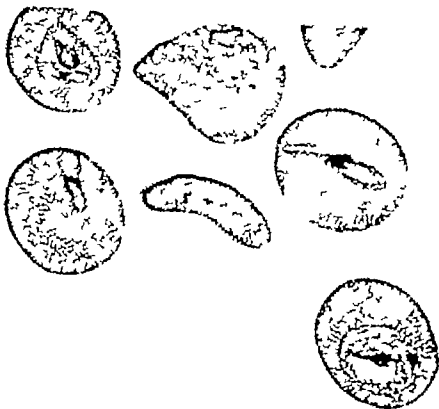


FIG 5

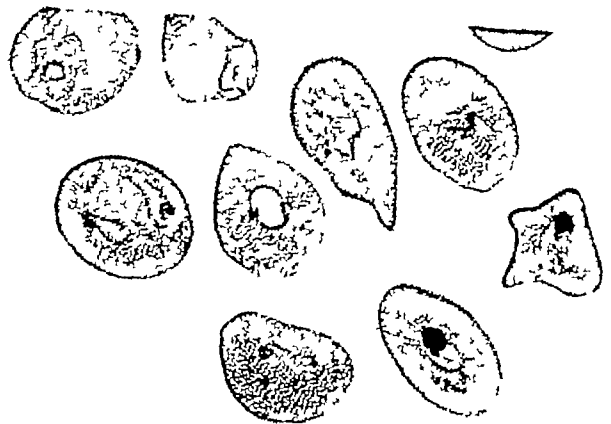


FIG 6

In column B are shown those cells which in malaria gave a positive peroxydase reaction, and the markedly wide variation in the percentages of these cells is, in all probability, due to the failure on the part of the writer to discriminate between those cells which were peroxydase reacting in virtue of having ingested positive material, and those which gave a true peroxydase reaction. When it is remembered that, at certain stages of the disease, e.g., after sporulation of the parasite, malarial blood contains large quantities of cellular and other debris, it is conceivable that some of the mononuclear phagocytes which would normally give a negative peroxydase reaction might, under these circumstances, give a positive reaction after ingesting positive reacting material.

## IX SUMMARY

Attention has been drawn to a few of the more important observations having a bearing on mononucleosis and leucopenia, the two most constant changes in the peripheral blood in malaria. Some definitions of the mononuclear cells described by the older authors have been quoted. Detailed descriptions of some of the more recent methods of investigating the mononuclear cells of the peripheral blood have been given. An attempt has been made to co-relate the various terms which have from time to time been used in connection with these cells (*vide* Table I). The results of two series of experiments on the nature of the mononuclear cells of the peripheral blood in malaria, carried out by some of the more recent methods, have been shown in Tables II and III, and the significance of the findings discussed.

## X CONCLUSIONS

Analysis of the results of these experiments indicates that the following are the probable changes produced in the mononuclear phagocytes of the blood as the result of malarial infection, but the number of cases examined is not considered sufficient to enable any definite conclusions to be drawn.

(1) There is an increase above normal of the R E cells in the peripheral blood in both acute and chronic malaria.

(2) This increase is due to the presence of mononuclear cells of different types.

(3) In acute malaria both clasmatoocytes N R (D), and monocytes N R (R), are markedly increased (possibly due to the greater number of cases in this group with enlarged spleens).

(4) In chronic malaria the clasmatoocytes are increased, but to a lesser extent, while the increase in the monocytes is similar to that in acute cases.

(5) In both acute and chronic malaria there is a variable number (usually the majority) of those mononuclear cells which give a positive peroxydase reaction with the Sato and Sekiya technique.

In conclusion I should like to express my gratitude to Major J. A. Sinton, V.C., O.B.E., I.M.S., Director, Malaria Survey of India, Kasauli, not only for the suggestions leading to this inquiry, but also for the help and encouragement which



he has given me in carrying out this research I am also indebted to Lieutenant-Colonel W T McCowan, I M S, Commanding, Indian Military Hospital, Dehra Dun, and to Major S Smith, R A M C, Officer-in-charge, Malaria Treatment Centre, Kasauli, for their kindness in placing clinical material at my disposal, and to Captain P F A Grant, I M S, Officer-in-charge, Brigade Laboratory, Dehra Dun, for kindly providing me with laboratory accommodation

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# ON SOME OBSERVATIONS ON THE MALARIAL PARASITES GROWN AEROBICALLY IN SIMPLE CULTURES WITH SPECIAL REFERENCE TO THE EVOLUTION AND DEGENERATION OF THE CRESCENTS \*

BY

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CULTURES whether obtained by the original technique of Bass and John(1), or by that simplified by the author of this memoir(2), or by the more elaborate and complex modification of the same described later by Sinton(3), yield only one single first crop and no satisfactory sub-cultures. Therefore all cumbrous details of the older technique(4) have been done away with in the present study, a procedure which has yielded quite satisfactory results both in point of the quantity and morphological details of the developing parasite in culture (Plate LXXXVIII, figs 1 and 2)

The facts gathered from such a study are as follows —

1 The great variation in the number of merozoites yielded by schizogony of *Plasmodium vivax*, *Plasmodium falciparum* and *Plasmodium malariae* this depending on (a) the nature of the infection, (b) the stage of the infection, (c) the number of attacks the patient had during the same infection, (d) the general clinical condition of the patient when the infecting material is collected, and (e) the biochemical properties of the serum used for culture (Plate LXXXVIII, figs 2, 3 and 4)

2 The fate of the merozoites produced and their rôle in the evolution of the crescent and gamete(5)

3 The evidence of antibody formation in the course of a suitable infection

4 The application clinically of (3), by a vaccine in reducing rapidly the number of crescents in the circulating blood

5 The study of factors contributing to failures of culture

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\* This work was done under the auspices and with the aid of a grant from the Indian Research Fund Association (1928-29)



# PLATE LXXXIX

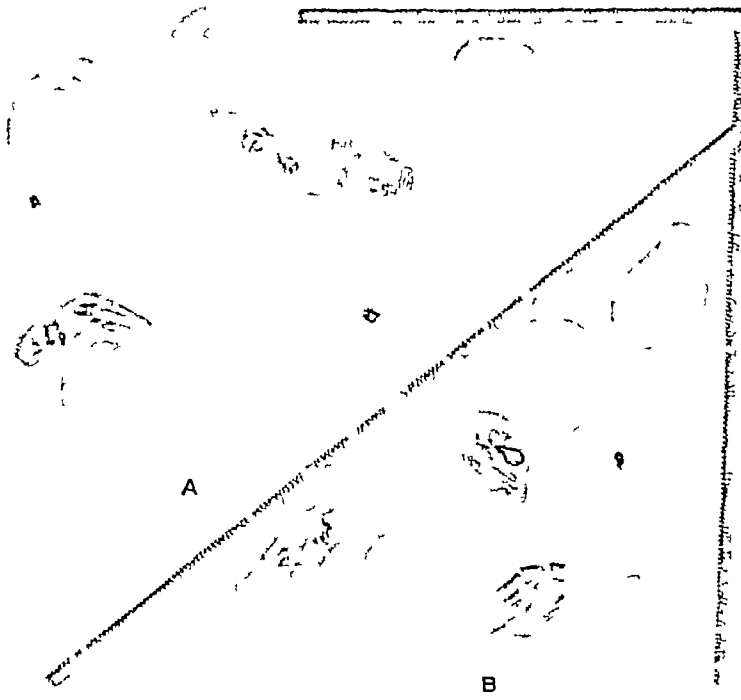


FIG 1



FIG 2

FIG 3

FIG 4

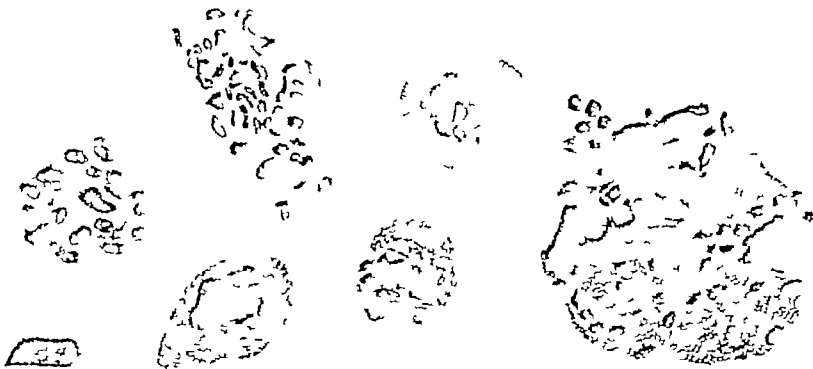


FIG 5

Leaving aside the obvious variations in the number of merozoites in the different species of the infecting parasites and the viability and the number of the parasites when manipulated for culture, there are certain definite variations observed in the same parasite during the different attacks of the same infection. Thus, when a man fresh from Europe and otherwise healthy is infected, one may find in his first paroxysm a very severe clinical reaction and yet the number of parasites in his peripheral blood may be very few. These, however, yield a large number of merozoites in culture, whereas during the second and third paroxysm, though the number of parasites in his peripheral blood may be very large, the merozoites yielded in culture is much smaller, and this diminution progressively continues for each paroxysm until a time is reached when one merozoite entering a red blood corpuscle produces no more than one individual. This is the point when the crescent and gamete production is initiated and once started it continues so as ultimately to flood the blood with these resistant forms of parasite which, as we all know, persist for several weeks during the apparent well being of the patient.

The factors contributing to the change in the direction of the parasitic development have been observed to depend (a) on the vigour of the phagocytes and (b) on the biochemical action of the plasma or serum in which the free merozoites find themselves either after the rupture of the fully developed schizont or after their escape from the phagocytes or on (a) and (b) combined.

### *Phagocytosis*

In culture the merozoite, once inside a red blood cell, continues its development uninterruptedly even in the midst of leucocytes and these seem to wait 48 to 72 hours or more until this full maturation is reached (Plate LXXXVIII, fig 3) when all of a sudden there is a chemotaxis followed by intensive phagocytosis (Plate LXXXVIII, fig 4). During this process the leucocytes degenerate, liberating such of the merozoites as have survived the phagocytic destruction, which in cultures are now acted upon by blood serum and are mostly plasmalysed (Plate LXXXVIII, fig 5). *In vivo*, however, these liberated merozoites are probably in a position to attack fresh red blood corpuscles and initiate a fresh paroxysm. The merozoites during each succeeding paroxysm, though presumably more resistant owing to their passage through the leucocytes, are found to lose their capacity to multiply until the point above referred to is reached when one merozoite yields but one individual for one red corpuscle attacked and the crescent formation is inaugurated and continued. These observations are supported by a simultaneous study of the films made from peripheral blood side by side with those prepared from cultures of the material gathered from the same patient during the successive attacks of the same infection or during the later attacks of different patients by the same parasite.

### *Secondary Anæmia*

Crescents make their appearance in the anæmic state so characteristic of repeated malarial attacks, provided it is not hydræmia because in such a thin medium

#### DESCRIPTION OF PLATE LXXXIX

- Fig 1 Evolution of the crescent, no schizogony A 24 hours B 48 hours  
„ 2 Schizogony of the parasite, 2nd relapse *N B* —Number of spores  
„ 3 Schizogony of the parasite of the same patient, 4th relapse *N B* —Diminished number of merozoites  
„ 4 Schizogony of the parasite of the same patient the fate of the parasites after immunization no development even after 48 hours in culture  
„ 5 Plasmolysis of the merozoites under the influence of the serum and phagocytosis

poor in proteins the parasites not only do not grow but as a rule degenerate and macerate whether they are inside or outside the pale vacuolated red blood cells

### Other Concomitant Clinical Facts

The other clinical findings usually met with in such cases are a well marked asthenia out of portion to the anæmia and a low blood-pressure with increased glucose content indicating a possible hormone insufficiency The following table illustrates these phenomena —

TABLE I.

Expt and date	When infected and nature of infection.	Cultural characters	Merozoites	No of crescents in 25 c mm	Date of observation	Blood-sugar Per cent	Blood-pressure	Total R B C in 1 c mm and Hb per cent
110 16-7-28	M T Rings B T. advanced forms Apyrexia of 19 days ago	Auto rich M T. culture B T gametes	6 to 8	32	18-7-28	0.13	90 (S)	3.9 ml 60 per cent
				8	31-7-28		55 (D)	
				0	7-8-28			
111 20-7-28	M T Rings 2 months	Auto very few advanced forms Diminishing every day But crescents increasing	8 to 12	6,383	22-7-28	0.129	85 (S)	4.4 ml 79 100 C I 68 per cent
				1,120	4-8-28		55 (D)	
				0	13-8-28			
113 3-8-28	M T Rings 4th attack 4th parox	Auto normal growth Hetero Anæmic serum Culture degenerated	5 to 13	18,570	13-8-28	0.118	90 (S)	5 ml 65 per cent 65 C I 100
				640	18-8-28	0.123	55 (D)	
				229	23-8-28			
				0	25-8-28			
114 4-8-28	M T Rings and crescents 8th attack 3rd parox	Auto no merozoites but pre-crescents and crescents	0	105,800	7-8-28	0.137	86 (S)	3.7 ml 50 per cent
				2,181	15-8-28		55 (D)	
				0	27-8-28			
115 17-8-28	M T Rings 6th attack	Auto normal growth Hetero quinnized serum agglutination and no further development	3 to 5	219	28-8-28	0.166	96 (S)	4.8 ml 80 per cent
				0	4-9-28		70 (D)	
116 28-8-28	5th attack 1st parox M T Rings only	Auto no Schizogony, crescents developed	0	121	30-8-28	0.123	90 (S) 55 (D)	5 ml 65 per cent

# DYSENTERY IN THE LAHORE MILITARY DISTRICT

BY

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[Received for publication, December 8, 1928]

PRIOR to the year 1925, dysentery amongst the troops in India was regarded as being almost entirely of amoebic origin, statistics up to that year showed only a very small percentage of cases amongst the troops to be due to bacillary dysentery

It has long been recognized, on the other hand, that dysentery in certain sections of the civilian community, notably in the population of certain Indian jails, was chiefly of bacillary origin. For example, Cunningham and King, writing in the *Indian Journal of Medical Research* in 1917, described 86.5 per cent of the cases of dysentery in the jails of Eastern Bengal as being of bacillary origin, and only 7.7 per cent as being due to the amoeba of dysentery.

That these relative proportions of bacillary and amoebic dysentery were not confined to jail populations was shown later by Cunningham, and again in 1924 by Acton and Knowles, working amongst the civil population in Calcutta.

The work and writings of these officers, however, appear to have had little effect on the bulk of the civilian practitioners throughout India or on the military medical officer.

In the case of the latter, the question has been carefully investigated during the last 3 years in all parts of India where troops are stationed. Manifold, working in Poona in 1925, proved that the vast majority of dysentery cases amongst the troops there was due to *B. dysenteriae* Flexner and that the exceedingly common 'Poona-itis' was of the same origin. In 1925, specialists in pathology, trained in the R. A. M. College, London, were appointed to the military laboratories in India and since then it has become apparent that the vast majority of dysentery cases amongst the troops in India are of bacillary origin.

Table I shows how the change has taken place in Lahore district. In 1924, for example, and to some extent in 1925, the total dysentery in the Lahore district was described as almost completely protozoal. A certain amount of

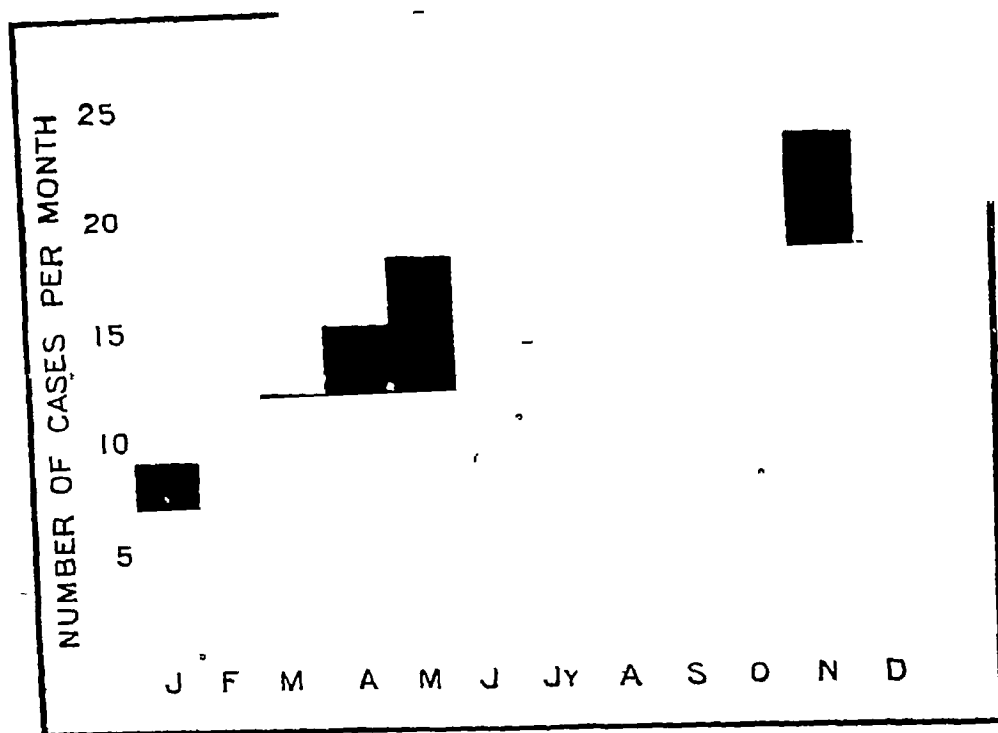
TABLE I—concl'd

Expt. and date	When infected and nature of infection.	Cultural characters	Merozoites	No of crescents in 20 c mm	Date of observation	Blood-sugar Per cent.	Blood pressure	Total R B C in 1 c.mm and Hb per cent
113	No crescents			0	4-9-28			65 C I 100
117 28-8-28	9th parox M T Rings Quinine given	Auto normal growth Hetero very rich in leucocytic blood serum	3 to 8	158 0	3-9-28 10-9-28	0 156	92 (S) 56 (D)	3 1 mil 55 per cent
118 5-9-28	Ditto.	Auto contaminated		1,470 210 0	11-9-28 19-9-28 25-9-28	0 086	115 (S) 80 (D)	3 8 mil 70 per cent.
119 12-9-28	11th parox M T Rings Quinine given	Auto normal culture Hetero quinnized culture, degenerated	3	1,419 587 0	17-9-28 25-9-28 2-10-28	0 143	95 (S) 60 (D)	3 1 mil 60 per cent
120 13-9-28	13th parox M T Rings	Auto normal Hetero in Diabetic serum — negative	3 to 15	913 87 0	25 9-28 2-10-28 9-10-28	0 181	98 (S) 60 (D)	2 4 mil 50 per cent
127 7-10-28	Crescents only One month fever Parox.?	No Schizogony	0	714 59 0	7-10-28 9-10-28 17-10-28	0 134	85 (S) 50 (D)	2 8 mil 60 per cent
128 13-11-28	Crescents only 16th parox No of attacks?	Auto no cultural development	0	918 86 0	13-11-28 21-11-28 26-11-28	0 206	86 (S) 60 (D)	3 5 mil 50 per cent
129 29-11-28	M T Rings and crescents 6th parox. Off and on fever	Auto normal culture and pre-crescents,	3 to 12	28,000 Vaccine 8,000 2,500	4-12-28 6-12-28 10-12-28 13-12-28	0 166	95 (S) 60 (D)	3 5 mil 75 per cent

NOTE—The alienation B T means infection with *Pl. vivax* and M T. infection with *Pl. falciparum* Auto means culture in autogenous serum, hetero means culture in heterogeneous serum, (S) and (D) mean systolic and diastolic pressure in mm of Hg

dysentery in 1924 (20 per cent) and in 1925 (6 per cent) was said to be clinical and is not shown in Chart 1

CHART 1



In 1926 and 1927, after the re-organization of the military laboratories, it was found that the position was almost completely reversed. Only about a quarter of the dysentery could be said to be protozoal, as over 76 per cent of it was very definitely bacillary, the causative organism being isolated and proved serologically in the majority of the cases.

The main reasons for this change in the diagnosis of dysentery amongst the troops are —

- 1 It became recognized that specimens from dysentery cases had to be dealt with immediately, and that if they could not be examined and cultured immediately, it was essential to preserve the material for examination so as to keep the organisms from being overgrown. Teague's glycerin and saline fluid was taken into general use for this purpose and proved most valuable.

- 2 The use in laboratories of litmus lactose-agar plates and the picking off of some half dozen colonies from each plate, the washing of the mucus in several changes of saline before plating, and in fact the adoption *in toto* of the methods of diagnosis taught by the R. A. M. College was the chief factor in bringing about the change.

- 3 Medical officers became chary of diagnosing 'dead amœbæ' when it is shown that the 'dead amœbæ' or 'cysts' were in very many cases nothing more than phagocytes or polynuclear pus-cells. Acton and Knowles' pamphlet on

*The Development of the Crescent in Culture*

The ring begins to shrink, the vacuole disappears in 24 hours and all the cytoplasm is reduced to a mere shell round a solid dot of chromatin, the hæmoglobin of the red blood corpuscle becomes finely granular and rapidly absorbed by the parasite, now growing and separated early as hæmozoïn, leaving the infected corpuscle much paler than before, the parasite with its cytoplasm now enlarges elongating in its growth, sometimes remaining straight and at other times slightly bent. The chromatin becomes woolly and gathers round the centre and the separated hæmozoïn is deposited in fine granules at both its poles and the crescent is complete in 48 hours, the body of the infected corpuscle being now reduced to a mere shadow of itself and made out as a pale pellicle characteristic of its appearance in stained blood films (Plate LXXXVIII, figs 5 and 6 and Plate LXXXIX, fig 1)

That antibodies are formed in a malarial infection is proved by the fact that in a certain number of cases otherwise healthy the infection comes to an end *per se* without any specific antiparasiticide. In cultures this fact is demonstrable by the lysis undergone by the merozoites when they are set free either by the direct bursting of the mature schizont or after their escape from the destruction by the leucocyte (Plate LXXXIX, fig 4). The ultimate disappearance of the crescents is also probably due to the production of an antibody, although in nature it takes several weeks, 3—7 weeks or longer (Sinton), 12 weeks (Darling).

On this hypothesis an attempt made to destroy the crescents by means of a vaccine prepared from the crescents gathered from suitable cases has yielded some striking results and the following few figures may be justifiably quoted as a preliminary observation —

TABLE II

Case No	Date of 1st appearance of crescents	Auto-vaccine date	Date of disappearance	Number of crescents and gametes with oöte Per 25 c mm of blood				
103	29 5 28	30-5-28 6-6-28	26-6-28	1,628 29-5-28	1,276 5-6-28	648 12-6-28	67 19-6-28	0 26 6-28
108	21-6 28	5-7-28	9-7-28	96 gametes		0 9-7-28		
110	18-7-28	24-7-28	31-7-28	32 crescents and some gametocytes		8 crescents 30-7-28		0 7-8-28
111	22 7 28	29-7-28 9-8-28	13-8-28	6 383 22-7-28		1,120 4-8-28		0 13-8-28
113	12 8 28	15-8-28	26-8-28	18,570 13-8-28		640 ? 24-8-28		? 
114	4-8 28	9-8-28 16-8-28	22-8-28	105,840 7-8-28		2,178 15-8-28		0 22 8-28
129	29-11-28	6-12-28	?*	28,000 4-12-28		8 000 10-12-28	2 100 13-12-28	0 ? 

\* Expt is in progress

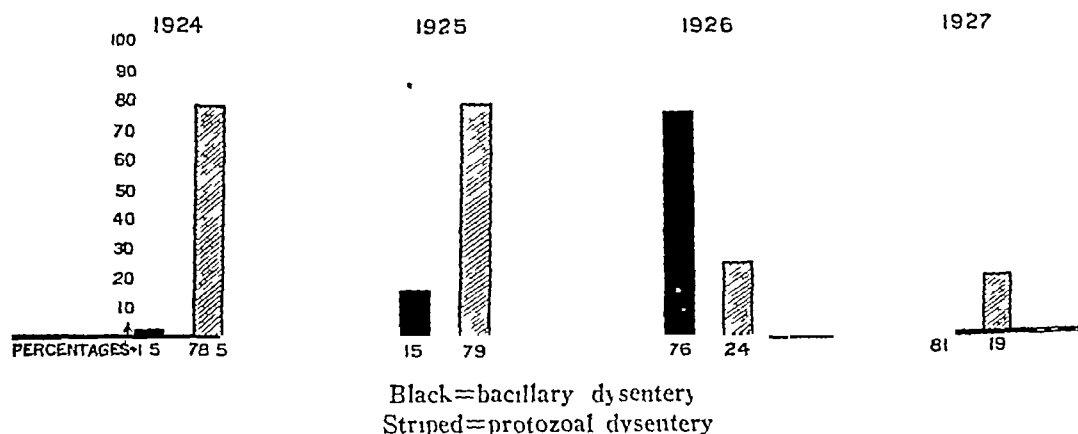


dysentery, supplied to every M O in 1925, with its pictures of bacillary and protozoal exudates, pointed out the distinctions clearly and aided greatly in more accurate diagnosis than was possible before 1925

### *Seasonal Incidence of Dysentery in Lahore District*

A study of the figures for the total dysentery cases in the Lahore district over a period of four years, 1924—1927, shows that dysentery cases increase markedly in incidence at two periods of the year, just before the hot weather, in March, April and May, and just after it, in September and October. These months are warm, but not as a rule unduly hot, and it is in these months that flies are most prevalent. If the average figures for total dysentery cases over the period of four years be considered as in Chart 2, it will be seen that there are two periods of comparatively low incidence. The first, January to February, coincides with the period when the weather is too cold for flies, and the second, June to July, when there are few flies because, presumably, it is too hot.

CHART 2



### *Method of Diagnosis*

The work of Manifold and of Acton and Knowles was followed, as closely as possible, throughout the investigation. In all cases a provisional diagnosis was first made as the result of a microscopical examination of the stool, with a view to enabling appropriate treatment of the case to be commenced immediately. The distinctive characters of the bacillary exudate when examined under the microscope are so well known that no description is necessary here.

Taking the results of one laboratory in 1927 as an example, 86 cases of definite dysentery were examined. Of these 74, or 86 per cent, showed the typical bacillary exudate consisting almost entirely of leucocytes and pus-cells, the remaining 14 per cent showing nothing very characteristic, unless, as in 7 cases, the *Amœba histolytica* was found. In this laboratory the percentage of bacillary cases is slightly larger than that shown in Table I, which includes the results of several laboratories.

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- (2) ROW, R (1917) On simplified technique of Bass's method of cultivating malarial parasites *in vitro* Ind Jour Med Res, IV, p 388, Pts XVIII—XXIV
- (3) SINTON, J A (1922) Simplified method for the cultivation of *Plasmodium falciparum* *in vitro* Ind Jour Med Res, X, p 203
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From the cases classed on microscopical examination of the exudate as bacillary, the causative organism was isolated in 69.1 per cent. The large number of bacillary cases in which the organism could not be isolated (30.9 per cent) is due to the fact that a large number of specimens examined in this laboratory come by post from out stations, preserved in 33 per cent glycerine saline, and some do not arrive until they are two or three days old. In all such cases, a provisional diagnosis is made on examination of a dried film of mucus and the result of this posted immediately to the hospital concerned.

### *Method of Culture*

A small piece of mucus washed in saline was placed on litmus lactose-agar. Next day a number of colonies were picked off and inoculated into lactose, one or two of the most likely being put into glucose and mannite at the same time. In this way a diagnosis as to whether the organism was of the Flexner or Shiga-Schmitz group, could usually be given on the biochemical and morphological appearances, on the third day after receipt of the specimen. The necessity of picking off and testing four or five organisms from each plate was often demonstrated, for on occasions, perhaps three colonies turned out to be *B. asiaticus*, only one or two giving the typical sugar reactions of *B. flexner*, etc.

### *Types of Dysentery Organisms*

The organisms isolated from bacillary cases during the period divided themselves on their biochemical reactions into three main groups, the Flexner group (74.1 per cent), the Shiga or Schmitz group (12.6 per cent) and the lactose fermenting group (13.3 per cent).

The main object of the investigation was to differentiate the Flexner group into its various sub-groups, which was done serologically as follows—

*Serological Tests*—High titre sera were produced in the usual way from rabbits inoculated with varying doses of *B. flexner*, V, W, X, Y and Z, obtained from the R. A. M. College, London. All these sera were preserved by the addition of glycerine 25 per cent to 50 per cent according to the titre obtained.

Cultures of each organism in Lemco broth, killed by 0.1 per cent formalin, were then tested by Dreyers' agglutination method against each of the high titre sera. Preliminary tests showed that the four hours' incubation advocated in the textbooks did not produce anything like the highest figure of agglutination possible. This was therefore lengthened to 8 hours, then to 16 and finally, for ease of working, the tubes were left in the water bath at 56°C overnight, receiving about 20 hours' incubation.

Tests of each unknown organism were on each occasion accompanied by a test of the known organism, i.e., the exact titre of the serum was estimated on each occasion an untyped organism was tested. This was found to be necessary as the laboratory is left unoccupied overnight, with a possibility, therefore, of

#### DESCRIPTION OF PLATE LXXXVIII

- Fig 1 24 hours culture of *Pl falciparum*, all parasites seen in one part of the field of the microscope
- „ 2 72 hours culture of *Pl falciparum*, all parasites seen in one part of the field of the microscope
- „ 3 48 hours culture of *Pl falciparum* showing chemiotaxis
- „ 4 72 hours of the same showing phagocytosis
- „ 5 Evolution of the crescent from one ring 24 hours culture
- „ 6 Evolution of the crescent from one ring 48 hours culture

the incubator lamp going out and shortening the incubation period. The agglutination figures shown hereafter as percentages of full titre are therefore strictly comparable with each other.

Each unknown organism tested was subcultured daily at least 10 times before testing.

Taking the results of testing one serum against its own and the other organisms of the Flexner group as an example, the effect of prolonging the incubation from 8 to 16 hours is shown in Table II.

TABLE II

Serum.	Organisms	8 hours' incubation		16 hours' incubation	
Type	Type	Fraction of full titre	Percentage of full titre	Fraction of full titre	Percentage of full titre
V	W	$\frac{125}{2,500}$	5	$\frac{250}{5,000}$	5
V	X	$\frac{250}{2,500}$	10	$\frac{500}{5,000}$	10
V	Y	$\frac{1,000}{2,500}$	40	$\frac{1,000}{5,000}$	20
V	Z	$\frac{125}{2,500}$	25	$\frac{250}{5,000}$	5

It will be noted that *B. dysenteriae* Flexner Y agglutinates with the 'V' serum to 40 per cent of titre after 8 hours' incubation, but to only 20 per cent on incubation for 16 hours. Exactly similar instances occurred when testing other stock organisms against the stock serum, e.g., *B. dysenteriae* Flexner X against 'W' serum agglutinated to 25 per cent of full titre after 8 hours' incubation and to only 10 per cent on 16 hours at 56°C (not shown in table). One gathers from this that the time necessary for development of full agglutination varies with each organism. This is well shown in Table II where, in the test of serum V against Y organism, the Y organism agglutinated to its full extent (1 in 1,000) in 8 hours, but the V organism took 16 hours to agglutinate fully (showing 1 in 2,500 at 8 hours, and 1 in 5,000 at 16 hours).

Many tests showed that no further agglutination occurred after more than 16 hours' incubation.

During the period occupied by the enquiry, 100 organisms of the Flexner group were collected and examined. These were placed in the following groups as the result of serological tests.

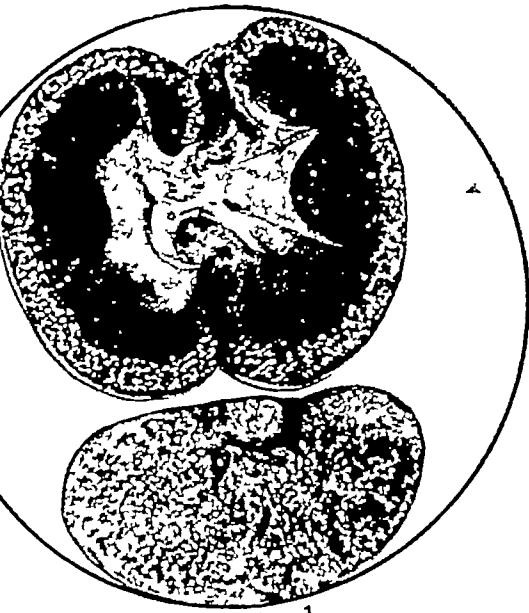


Fig 1

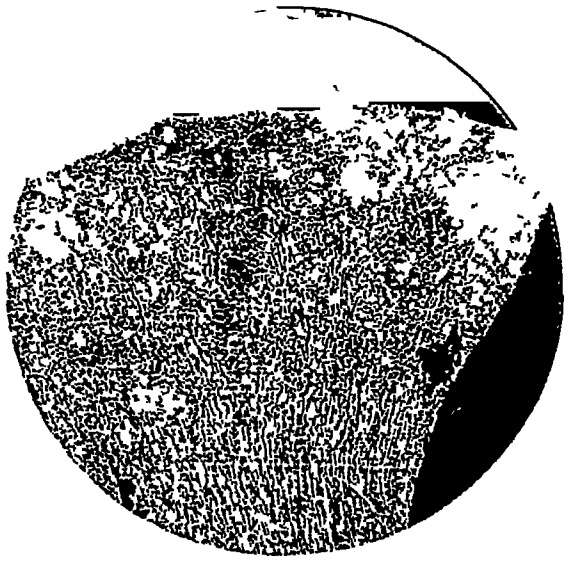


Fig 2

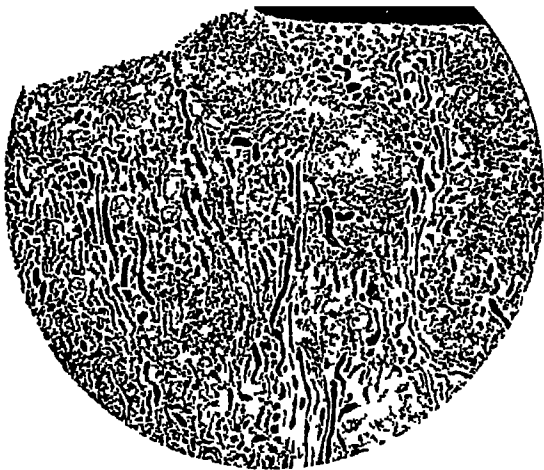


Fig 3



Fig 4

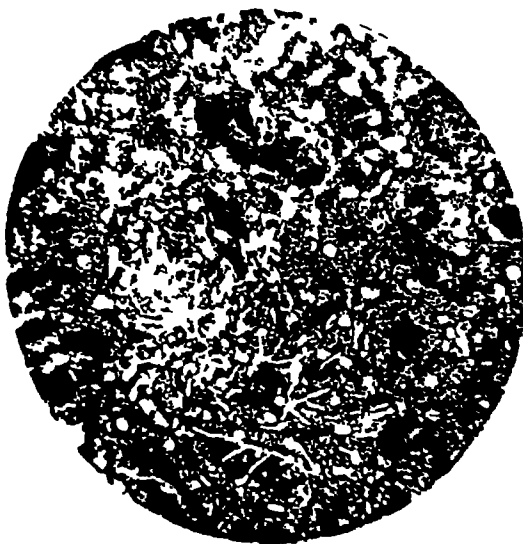


Fig 5

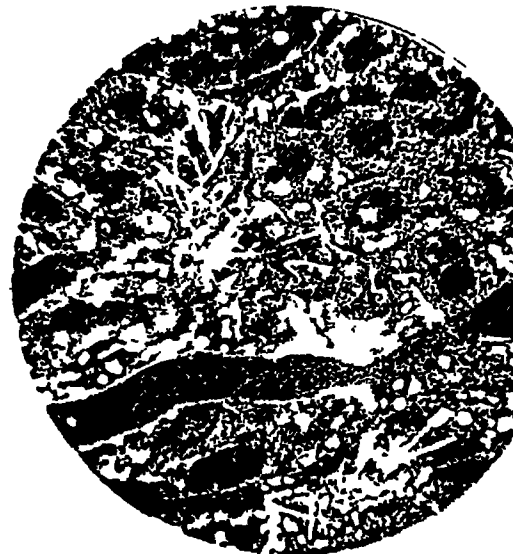


Fig 6



Agglutination of the organism to 100 per cent of the titre of the type serum was considered necessary for 'typing' purposes

1	<i>B. dysenteriae</i>	Flexner	V	4 per cent
2	"	"	W	36 "
3	"	"	X	8 "
4	"	"	Y	3 "
5	"	"	Z	11 "
6	"	"	VZ	4 "
7	"	"	,, (imperfectly agglutinable type) 34 "	

From this it will be seen that *B. dysenteriae* Flexner W is the commonest of the Flexner organisms isolated, occurring in this series to the extent of 36 per cent. It is to be noted that the sera used for typing this series were all made from organisms obtained from the R A M College, London, and the results are therefore comparable with those published in 1927 by Manifold working in Poona and Wats and Loganaden in Secunderabad and also with those of Little in Mhow, who obtained their type cultures from the same source. The results obtained with this Lahore series correspond very closely with those noted in other parts of India and do not differ markedly from those obtained by Wolff working in Deli (Dutch East Indies). Many of the results noted in the series were controlled by agglutination experiments (*see later*).

*The imperfectly agglutinable group*—This group occupied no less than 34 per cent of the total number of organisms classified as *B. dysenteriae* Flexner by their carbohydrate reactions. On first isolation of the organisms, this group appeared to be divisible into two sub-groups.

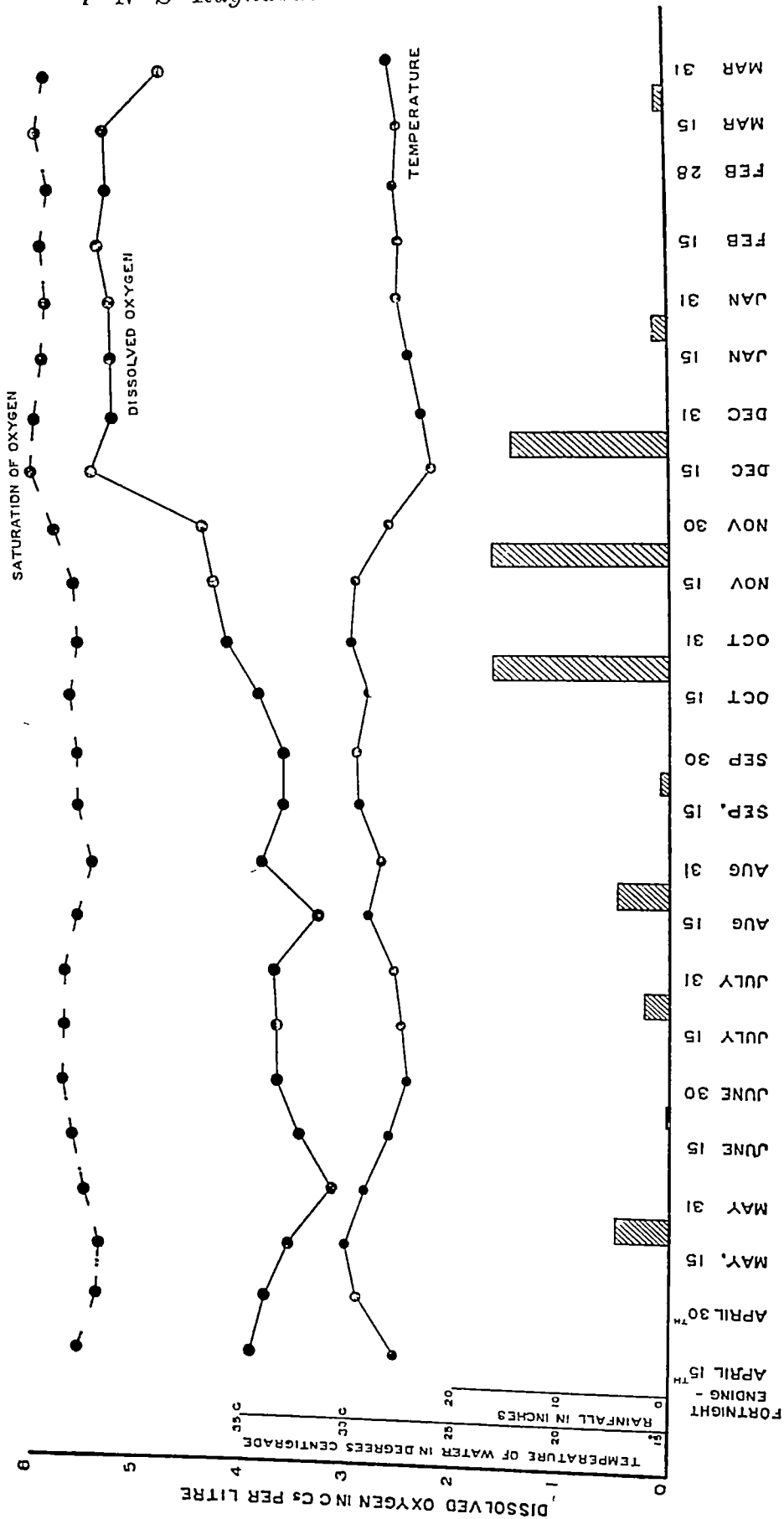
(a) The first sub-group, consisting of 16 per cent of the total Flexner organisms, could not be said to agree with any of the named types (V, W, X, etc.), for the reason, in the majority of cases, that the organisms did not agglutinate with any of the type sera to a sufficiently high titre. The exact titre of the serum used was estimated on each occasion, and those which did not agglutinate with any of the type sera over 50 per cent of full titre were placed in this group. This group was tested on three occasions, e.g., at the 10th, 15th and 20th subculture, but no significant difference was found in the results. Further work is required on this group.

(b) The second sub-group, consisting of 18 per cent of the series, appeared at first to be completely magglutinable with any of the known types of Flexner sera. Tests of this group were made on many occasions and for many months no change was found in their characteristics.

From four of them high titre sera were prepared in the usual way and in no case was it possible to obtain a higher titre than 1 in 250, whereas, using the same method, a titre of 10 times this amount could easily be obtained with the ordinary Flexner organisms. This agrees with Manifold's finding in Poona. Even this titre fell very rapidly on keeping, so that at the end of a few months, it was seldom more than 1 in 50.



CHART I



Attempts were made to produce a higher titre in three of the cases by using Susman's method of injecting solution of brain extract subcutaneously, concurrently with the intravenous injections of the organism, but no appreciable difference was found in these cases

On the other hand, an apparently higher titre could be produced by following Formicola's method of using a culture of the organism in taurocholate broth instead of ordinary broth. Ordinary broth was used in the tests which follow, as sometimes false agglutination occurs with taurocholate broth

The result of testing three of these sera against their homologous, and certain heterologous inagglutinable Flexner organisms is shown in Table III. The organisms used in this test were between three and four months old, and from the result of the test, it might be said that three seem to belong to one serological group, e.g., Adjudia Lal, Smith and Sujan Singh, and that two others, Hodder and McLellan seem to belong to another serological group. On the other hand the inagglutinable B Dymott produced a serum which, when injected into a rabbit, agglutinated *B. dysenteriae* Flexner Z to 100 per cent of titre. It would seem therefore that this organism although inagglutinable is really a 'Z'.

TABLE III

Organisms	SERA (Made by Susman's method)							
	Adjudia Lal	Hodder	Dymott	B FLEXNER				
				V	W	X	Y	Z
Adjudia Lal	1 in 125							
Smith	100 per cent							
Hodder		1 in 250						
Dymott			1 in 250					
McLellan		100 per cent						
Sujan Singh	200 per cent	10 per cent						
B Flexner V								
" W								
" X								
" Y			20 per cent					
" Z			100 per cent					

A serum made from B Smith proved identical with that made from B Adjudia Lal quoted above.

in the course of his investigations on the consumption of oxygen and of oxidation processes in sand filters, found 'the change in the dissolved oxygen content of water in passing through sand filters was greater than the changes in the absorbed oxygen' He states that the oxygen may have been used up either by the bacteria or by chemical reaction in the filters Gallaher in Michigan, U S A, experimenting with rapid filters found that there was a gradual increase in the amount of oxygen removed from the water by the filters as the temperature of the applied water increased

In the course of our observations, we also noticed the fact that slow sand filters produced practically no sulphuretted hydrogen during the two or three months following heavy rains, viz, December to March The dissolved oxygen content of the applied water at this time was, as already stated, round about the saturation point This finding of ours is in agreement with the experiences of other observers in America, notably those of Waring of Ohio and of Mahlie of Texas Waring states 'the decrease of the dissolved oxygen constituent is the most important single factor entering into the intensity of tastes and odours' From his experience at Fort Worth, in Texas, Mahlie concludes that 'when the dissolved oxygen in the raw water is around 50 to 60 per cent saturation, we have noticeable tastes When it is near 80 per cent, the taste is slight and when it reaches 95 to 100 per cent, there is no taste In the aeration of water several factors should be considered These are

- (1) the exchange and removal of obnoxious gases It goes without saying that the ideal is that which breaks the water into the finest particles, due allowance being made for wind action to dissipate the removed gases,
- (2) chemical and biological oxidation are necessarily slow and the time factor is of importance,
- (3) in securing the maximum biological action the logical place to aerate is before filtration, since organisms of various sorts enter this action'

In the light of our findings and of the experience of other observers, it is suggested that conditions similar to those obtaining at the bottom of a lake are established in the filters fed by water deficient in oxygen, and the evolution of sulphuretted hydrogen is the result of the anærobic putrefactive processes

Experiments in progress since October 1927, at Kilpauk under the direction of the Government Committee on Water and Sewage purification (of which the senior author is a member), have shown that pre-aeration of the Red Hills lake water through a percolating filter designed by Mr Westerdale, Sanitary Engineer to the Government of Madras, has resulted in totally eliminating, *even during the hottest part of the year*, the production of sulphuretted hydrogen when such pre-aerated water was applied to a sand filter The effluent from the secondary filter of this suite was of a uniformly excellent quality

The organic matter present in the lake water, as judged by the figures for oxygen absorbed in 4 hours at 28°C, by potassium permanganate (Tidy's

Further work on this sub-group, carried out about a year after the original isolation, considerably modified the original findings. Of the six organisms quoted above, only two were now completely inagglutinable, Adjudia Lal and Smith, and even these two could be shown by agglutination tests to belong to the 'X' type of *B. dysenteriae* Flexner. The results obtained on testing these six originally inagglutinable organisms after retention for a year in the laboratory are shown below —

Organisms	SERA				
	V	W	X	Y	Z
Adjudia Lal					
Smith					
Hodder	1 in 50	1 in 50			
Dymott					1 in 50
McLellan		1 in 25			
Sujan Singh			1 in 250		

Serum made from the year old organism 'Adjudia Lal' now agglutinates *B. Flexner* X to 10 per cent of full titre, while serum made from the organism 'Smith' agglutinates *B. Flexner* X to 100 per cent of titre. Comparing this with the table above, it will be seen that the three organisms which were thought originally to belong to the same group of 'inagglutinables', i.e., Adjudia Lal, Smith and Sujan Singh, really seem to belong to the 'X' type of Flexner organism.

Similar results have been obtained with six other originally inagglutinable Flexner strains, which have been more recently isolated, and in the case of two of them the property of agglutinability appeared about three months after isolation.

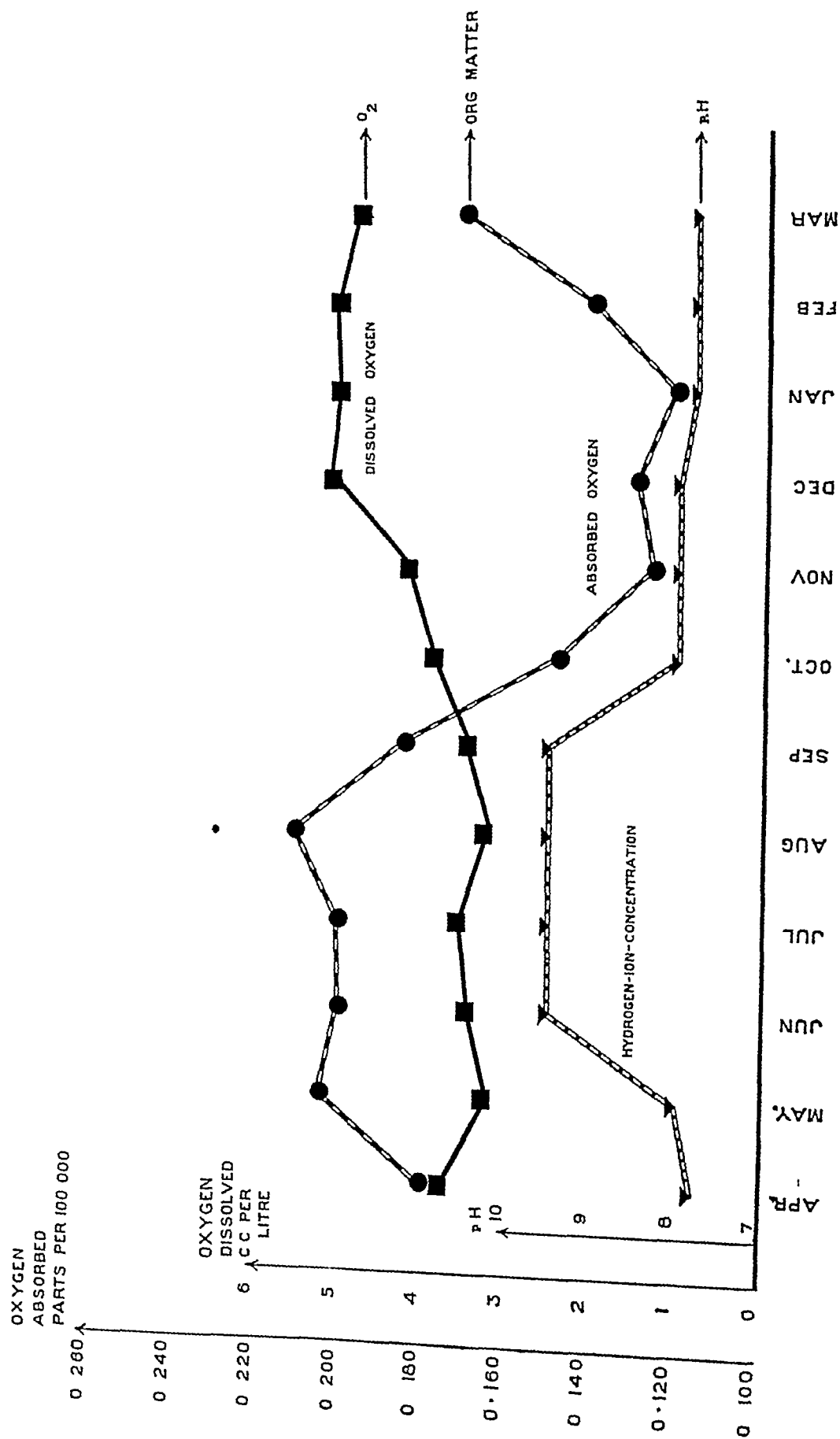
Certain others, less than three months old, remain completely inagglutinable with any of the Flexner type sera.

It would appear, therefore, that there is no true completely inagglutinable type of Flexner organism and that strains considered to be inagglutinable, will, if kept long enough, show a certain and possibly an increasing amount of agglutinability with specific sera.

Similar tests have not been carried out up to the present on the organisms placed above in the first sub-group of imperfectly agglutinable Flexner organisms, but possibly on keeping, these might develop sufficient agglutinability to enable them to be placed in one of the named types of *B. Flexner*.

From the point of view of the preparation of a prophylactic vaccine, the existence of this imperfectly agglutinable group does not perhaps matter much.

CHART II



More than half of the organisms isolated agglutinate with V, W, X, Y and Z sera to amounts varying between 10 and 50 per cent. It appears probable, therefore, that the remainder, even those found at first completely inagglutinable, will develop sufficient agglutinability or agglutinogenetic power to enable them to be placed in one or other of the named groups of Flexner's bacillus.

The composition of a prophylactic vaccine for dysentery would appear therefore to require the following —

1	<i>B dysenteriae</i> Flexner	V	4 per cent
		W	36 "
		X	8 "
		Y	3 "
		Z	11 "
		VZ	4 "
2	<i>B dysenteriae</i> (lactose fermenting)		
3	<i>B dysenteriae</i> Shiga and		
4	<i>B dysenteriae</i> Schmitz		

Theoretically such a vaccine would be protective against about 80 per cent of the total dysentery in India.

*The lactose fermenting group*—The results of preliminary work on this group have already been submitted for publication in the *Journal of the R A M Corps* under the heading of 'Notes on Sonne Dysentery in Lahore District'. This was carried out at the expense of the greatly increased monetary allotment now made to military laboratories in India. It attempts to show that some 12 or 13 per cent of the total bacillary dysentery in India is due to *B. Sonne*, which appears in India in two forms, an early form, showing circular smooth colonies, and a later development of this form, showing crenated irregularly spreading colonies, which develops after a varying number of subcultures of the original smooth form. Both forms of colony are agglutinable with a serum made from the first form, while a serum made from the second, crenated form, will agglutinate only this form, and have no effect on the circular smooth form. This is important in view of the fact that the stock cultures maintained in laboratories from which sera are usually made are invariably of the crenated type, while it is becoming increasingly apparent as a result of recent work in this laboratory, that *B. Sonne*, on first isolation, assumes the circular smooth form in the majority of cases. This fact perhaps explains the irregularity of agglutination found by many recent observers of this organism.

*Agglutminogenesis Tests*—In order to confirm the results of the agglutination tests carried out in this research, many agglutminogenesis tests have been performed. For example, an organism said to be *B. dysenteriae* Flexner W as a result of agglutination tests is injected into a rabbit and a high titre serum is produced in the usual way. This serum is then tested against the various bacilli of the Flexner group. If it agglutinates *B. Flexner* W to the same titre as its homologous organism and at the same time does not agglutinate other

process), is graphically represented in Chart II. The dissolved oxygen and the pH curves are also plotted out. It will be seen from this chart that the organic matter in the lake water

(a) is always very high and resembles that in dilute sewage,

(b) is at its highest during the hottest months,

(c) is noticeably decreased after the heavy rains and remains low till the level of the lake begins to fall appreciably.

The dissolved oxygen during the time that the organic content is at its maximum shows the greatest deficiency and vice versa. There is thus a positive correlation between the organic matter and the dissolved oxygen on the one hand, and between the dissolved oxygen and the temperature of the water on the other. The pH is highest when the organic matter is at its maximum and falls when the lake water gets diluted.

In the course of our investigation we made an attempt to collect specimens of the plankton forms present in the lake water, as also the plants growing in the lake. Amongst the higher plants, *Chara* was found to be the predominant type in the lake and to grow in such abundance that the screens at the inlets to the roughing filter basins at the source and to the filters at Kilpauk got frequently choked up and had to be cleaned several times during the day. Some species of this plant are known to produce  $H_2S$  in abundance, after death and decay.

Amongst the algæ, both of the green and blue-green varieties, the lake contains representatives of every known genus, the predominant forms of the green variety being, *Asterionella*, *Synedra*, *Navicula*, *Fragilaria*, *Pandorina*, *Eudorina*, *Spirogyra*, *Mougeotia*, *Tetraspora*, and *Ulothrix*. The blue-green variety which is really the more troublesome one, from the point of view of water-works operation, is represented largely by *Oscillatoria*, *Phormidium*, *Anabaena*, *Nostoc*, and *Cælosphaerium*. There are several other species belonging to both varieties, present in the water in fewer numbers, which for want of adequate time and expert assistance, we have not yet been able to identify. The blue-green algæ, which are present in large numbers in the lake water, according to Jackson and Ellms, will produce 'pig-pen' odours and bad tastes when they die, owing to the decay of highly nitrogenous organic matter in which partially decomposed sulphur and phosphorus compounds play a large part.

More work in this direction is needed and the economic aspect of fresh water biology with reference to the lakes in India which supply drinking water will require to be studied at great detail, if perfection is to be attained in the æsthetic and sanitary qualities of our drinking water.

#### SUMMARY AND CONCLUSIONS

A systematic study of the water of the Red Hills lake both at the source and as it reaches Kilpauk, carried out for 3 years, has revealed the following interesting facts —

- 1 The temperature of the water varies between 24 and 31°C
- 2 Stratification takes place in the water as a result of the action of the tropical sun

members of the group to a similar titre, it can be said that the identity of the organism as shown by the agglutination test is confirmed by agglutinogenesis. So far, such tests have been carried out only on bacilli which are definitely inagglutinable with the ordinary V, W, X, Y or Z sera, or which show such definite agglutinability with these sera, that they were placed in one or other of the named groups of Flexner's bacillus. The results in the case of the definitely agglutinating organisms have been in all cases to confirm the results of the agglutination tests. In the case of 'inagglutinables,' however, the production of a high titre serum has shown in the three cases so far tested that the agglutination test is not sufficiently reliable to allow the classification of a group of true inagglutinable Flexner organisms. The three sera referred to, which were made from organisms originally inagglutinable, show a certain amount of agglutinating power over various bacilli of the Flexner group. This is confirmed by late agglutination tests, which show that two of these organisms, when kept for a year in the laboratory, show some agglutinability with V, W, X, Y and Z sera.

#### SUMMARY

1 Bacillary dysentery in Lahore military district is very much commoner than amœbic, forming some 80 per cent of the total dysentery.

2 Dysentery in Lahore district, while present throughout the year, increases markedly in incidence in the warm months prior to the real hot weather and immediately following it.

3 Bacillary dysentery has been almost invariably very mild in type in the years 1925 to 1927, even in the case of Shiga infections. Some cases of Flexner infection had only one day's diarrhoea with blood and mucus in the stools.

4 Bacillary dysentery groups itself in Lahore district into that due to *B. dysenteriae* Flexner 74.1 per cent, *B. dysenteriae* Sonne 13.3 per cent and *B. dysenteriae* Shiga or Schmitz 12.6 per cent.

5 The Flexner infections assume the following proportions —

1	<i>B. dysenteriae</i> Flexner	V	4 per cent
2	" "	W	36 "
3	" "	X	8 "
4	" "	Y	3 "
5	" "	Z	11 "
6	" "	√Z	4 "
7	" "	(imperfectly agglutinable)	34 "

6 Agglutination tests in the case of dysentery require incubation for 16 hours at 56°C. It was found convenient to leave them overnight in the water-bath.

7 The diagnosis of Sonne dysentery requires a high titre serum made from a circular smooth colony of this organism, or preferably two sera, one from the circular smooth early form and another from the crenated late form.



3 Algal growths consequently increase, die and settle to the bottom where they decay and set up putrefactive processes resulting in a depletion of the dissolved  $O_2$

4 This depletion, it is suggested, is responsible for the production of  $H_2S$  in the filters

5 Aeration of the water before applying to a sand filter eliminates the tendency to  $H_2S$  production

6 Algæ of the green and blue-green varieties are present in large numbers in the water as also *Chara*. This latter and some forms of the blue-green algæ are capable of producing  $H_2S$  when they die and decay

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8 An originally large group of inagglutinable Flexner organisms was found on retention for a year in the laboratory to have become agglutinable to a small extent with Flexner sera and then to have become capable of producing a serum, which agglutinated various bacilli of the Flexner group, in some cases to high titre

In conclusion I beg to thank Major J A Manifold, DSO, for much inspiration and help during this research

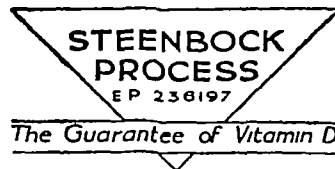
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# SOME OBSERVATIONS ON THE WATER OF THE RED HILLS LAKE AT MADRAS (1925—28) \*

BY

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[Received for publication, January 16, 1929]

THE water-supply to the city of Madras is derived from the Red Hills lake, which is about seven miles from the city. The lake has a catchment area of over 23 square miles and receives its main supply from a channel issuing from the Cholavaram tank, which is supplied by the Courtalliyar river through the Tamarappakkam anicut. The catchment area of this river and its tributaries comprises the Nagari hills of the Karvetnagar zamindary in the North Arcot district. The Red Hills lake thus receives water that has travelled over an extensive area and gathered a very large amount of polluting material, *en route*.

The city was supplied with the untreated lake water up till 1914 through a system of pipes emanating from Kilpauk, to which point the lake water was led through a raised open earthen channel. In December 1914, however, the new water-works were opened and the lake water was allowed to gravitate through a closed masonry conduit and filtered through a battery of 14 (since increased to 17) slow sand filters, before being supplied to the city. The present system of slow sand filtration has not, however, been the success that it was intended to be. The numerous investigations undertaken between then and now by various agencies, to find out the best means of purifying this water so as to render it thoroughly suitable for drinking purposes, go to show that the present system of slow sand filtration has not been able to produce a potable water.

The production of sulphuretted hydrogen in large quantities and the inability of the slow sand filters to produce at all times a water of a *uniformly good*

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\* Paper read before the Indian Science Congress, XVI Session, Madras, January 1929

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quality have been the two main drawbacks in the existing system. Further, it is not possible to chlorinate the filtered supply without inducing serious taste troubles, owing to the presence of sulphuretted hydrogen in the water. Towards the close of 1924, it occurred to the authors of this paper, that a systematic and detailed study of the raw water extending over a fairly long period was likely to throw some light on this important problem.

Accordingly, daily observations on the raw water reaching Kilpauk and fortnightly observations on the water at the source were started early in 1925. These included determinations of the temperature of the water, the depth of water in the lake, the dissolved oxygen content of the water at various points, and the nature and extent of the organic matter present. Data relating to rainfall and atmospheric temperature, as also to the hydrogen-ion concentration of the water, were also collected.

The passage of water through large impounding reservoirs like the Red Hills lake has long been known to bring about certain changes in the character of the water tending to improve its sanitary quality. Several agencies, e.g., sedimentation, dispersion and sunlight, as also lack of food material and possible oxidation, are usually responsible for the rapid disappearance of the pathogenic microbes introduced into a lake along with the flood water. Sunlight in the tropics acts very powerfully in bringing about a considerable reduction in the bacterial content of the water. At depths not penetrated by sunlight, the dissolved oxygen content is depleted by the decomposition of the organic matter. Subsequent putrefactive changes following the decomposition process, set up anærobic conditions at the bottom, which are inimical to the life of the pathogens, even if they had a chance to reach to the bottom. These are among the benefits derived from storage in lakes, but like many other good things, storage has its drawbacks and limitations.

In the first place, stratification takes place in the water, due to temperature differences between the top and bottom layers. This is very pronounced in the warm summer months, because

(i) the summer sun raises the temperature of the surface water by from 1.5 to 2.5 degrees centigrade, as compared with the bottom layers,

(ii) the lake receives no influx of fresh water during these months,

(iii) wind and wave action is at a low ebb during this time.

Consequently, the surface water is always warmer during summer than the bottom layers, and the stratification due to differences in density is so well defined that there is practically no possibility of the two layers mixing. The Red Hills lake is not a very deep one in the sense that the North American lakes (many of them are over 80 feet in depth) are deep. It has an irregular outline and a number of shoals and a few sandbanks. Its depth, when quite full (December and January), varies from about 25 feet at its deep to about 4 or 5 feet at its shallow regions. During the summer months the depths vary likewise from 8 or 9 feet to as many inches. In the three years comprised in the present enquiry, the maximum level of water in the lake was reached in December in 1925, and in

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January in 1926 and 1927 The lowest level in the same three years was reached in July of 1925 and September of 1926 and 1927

The temperature variations in the surface water of the lake from month to month are as under —

Month	TEMPERATURE	
	Minimum	Maximum
January	25.5°C	27.0°C
February	26.0°C	27.5°C
March	27.0°C	28.5°C
April	28.0°C	31.0°C
May	29.5°C	31.5°C
June	28.5°C	30.5°C
July	28.5°C	30.5°C
August	29.0°C	31.0°C
September	28.5°C	30.0°C
October	26.0°C	29.0°C
November	25.0°C	27.0°C
December	24.0°C	25.5°C

In the bottom layers, accumulations of organic matter are present at all times of the year, during the summer months they are particularly excessive and partake of the nature of black ooze. The addition of a few drops of a dilute acid to this ooze liberates sulphuretted hydrogen.

These accumulations are brought about partly by the washings loaded with organic matter brought in by the floods, but mainly by the algal and other vegetable growths which earlier grew on the surface, died and settled to the bottom. The bacterial decomposition and subsequent putrefactive changes, referred to in an earlier section of this paper, consume all or nearly all of the atmospheric oxygen present in the bottom layers. There is no possibility of the lower layers getting a fresh supply of oxygen for replenishment as there is always a stratum above, of water of lower density acting as a seal. The result is that the water in the deeper parts of the lake is charged with the products of anaerobic bacterial activity and is often found to be grossly deficient in its content of dissolved oxygen. The practice in Madras has been to draw off the water needed for the city supply from about three to five feet below the surface, depending upon the situation of the draw-off valve, with a view, it is stated, to avoid drawing surface impurities into the conduit going to the filters. Thus, water more deficient in its content of dissolved oxygen than



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the surface water is as a rule drawn for filtration. In our determinations, the surface water of the lake showed an average deficiency of from 7 to 14 per cent of the saturation needs of dissolved oxygen, the lowest figure was 1.2 per cent in December and the highest was 24 per cent in August. The dissolved oxygen content of the water from 5 feet below the surface when the lake was full showed a deficit of 12.5 per cent over the surface sample at the same spot, while a sample 8 feet lower down showed a further reduction in its oxygen of 7.5 per cent. In summer, when the lake was shallow, the deficiency was even more pronounced and was often over 30 per cent above the normal.

In its passage through the covered-in-conduit from the lake to the filter beds at Kilpauk, a distance of about 7 miles, the water was found to undergo a further depletion of its oxygen content as a result, probably, of the enormous growths on the side walls of the conduit utilizing a portion of the oxygen present in the water. The water reaching Kilpauk, thus depleted of its oxygen content, is applied to the slow sand filters, in the filtering layers of which further and much more serious depletion takes place and foul-smelling gases are generated.

Chart J gives the fortnightly averages for the dissolved oxygen figures, determined by the Winkler method, for the raw water at Kilpauk together with the temperatures and the saturation needs at those temperatures of the dissolved oxygen. The rainfall for each month is also plotted out on the same chart.

It will be seen from the above that (1) the dissolved oxygen content of the water has always been below the saturation needs, (2) the deficiency is greatest in May and again in August, (3) even a small fall of rain effects a noticeable increase in the oxygen content, (4) the dissolved oxygen reaches almost the point of saturation in December after the heavy rains, and (5) there is a definite correlation between the dissolved oxygen and the temperature curves, especially is this so in the summer months.

Having found that the raw water was deficient in oxygen throughout the year, we carried out a number of experiments on artificially supplying the deficiency by aerating the water under laboratory conditions. The water was aerated by pouring it out from one beaker to another at a constant height of 32 inches—the height of a laboratory table—for 2, 5 and 10 minutes. The results showed that in many cases two minutes' aeration was sufficient to bring the oxygen content to saturation, while in a few, particularly those with an initial deficiency exceeding 25 per cent, a five (occasionally ten) minutes' aeration was required to produce the same effect.

We next carried out a series of determinations on the raw and filtered water, from one of the slow sand filters functioning at a uniform rate of 4 vertical inches per hour, and found that the filtrate yielded a very much lower figure than the applied water, for dissolved oxygen even on the very first day after starting. There was a consistent and progressive reduction in the oxygen content of the filtrate from day to day and, when sulphuretted hydrogen appeared in the filtrate, the dissolved oxygen figure showed further progressive reduction and finally reached zero as the hydrogen sulphide increased considerably. Noll in Germany,

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## PROTOCOLS

### *List of abbreviations used*

K	<i>Monilia kruszewii</i>
M	'Minutus' type of monilia (a cryptococcus)
A	<i>Monilia psilosis</i> (typical)
Aa	" " (atypical)
S C	Subcutaneous inoculation
I V	Intravenous inoculation
I P	Intraperitoneal inoculation
G P	Guinea-pig
R	Rabbit
W M	White mouse
Mk	Monkey
P C	Peritoneal cavity
L I T C	Lister Institute Type Cultures
L I P M	Lister Institute of Preventive Medicine
H B	Heart blood
L <sub>iv</sub>	Liver
Spl	Spleen
K <sub>id</sub>	Kidney

PROTOCOL I  
*Animals which received a single intraperitoneal injection*  
 (1) Animals which died after the injection

Serial No	Animal number	Type and No of monilia strain *	Source of yeast	Died (days)	P M signs	Monilia found by culture				
						H B	L <sup>av</sup>	Spl	Kid	P C
1	155 GP	A 29	Sprue faeces	2	Acute peritonitis	+				
2	172 GP	A 29	Do	2	Acute peritonitis	+				+
3	209 R	K 605 (L I T C)	L I P M	5	Intraperitoneal lymph organized	—			—	
4	225 GP	A OXIII	Healthy faeces	2	Acute peritonitis	—			+	+

\* Type of yeast—The strain of yeast as described in our former paper 'Yeasts and Sprue'

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## PROTOCOL I (2) (contd.)

Serial No	Animal No	Type and No of monilia strain	Source of yeast	Killed (days)	P M signs	Monilia found by culture				
						H B	Lav	Spl	Kid	P O.
22	100 GP	Aa XX	Kala-azar faeces	14	Nil	—	—	—	—	—
23	109 GP	Aa XVI	Pernicious anæmia	24	Abscess P C	—	—	—	—	—
24	110 GP	Aa XVII	Diarrhoea non-sprue	24	Encapsuled abscess P.C	—	—	—	—	—
25	111 GP	K XVIII	Do	28	Nil	—	—	—	—	—
26	112 GP	K XIX	Do	28	Nil	—	—	—	—	—
27	113 GP	K XXI	Non sprue diarrhoea	28	Encapsuled abscess P O	—	—	—	—	—
28	130 GP	A 29	Sprue faeces	1	Acute peritonitis	+	+	+	+	+
29	140 GP	Do	Do	2	Plastic peritonitis	+	+	+	+	+
30	141 GP	Do	Do	3	Acute peritonitis	+	+	+	+	+
31	142 GP	Do	Do	4	Plastic peritonitis	—	—	—	—	—
32	143 GP	Do	Do	5	Acute peritonitis	—	—	—	—	—
33	144 GP	Do	Do	6	Plastic peritonitis	—	—	—	—	—
34	145 GP	Do	Do	7	Do	—	—	—	—	—
35	146 GP	Do	Do.	8	Nil	—	—	—	—	—
36	147 GP	Do	Do	9	Necrotic nodule P C	—	—	—	—	—
37	148 GP	Do	Do	10	Do	—	—	—	—	—
38	151 GP	Do	Do	20	Nil	—	—	—	—	—
39	152 GP	A 29 passed through GP	Do	2	Acute peritonitis	+	—	—	—	+

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Serial No	Animal number	Type and No of monilia strain	Source of yeast	Killed (days)	P M signs	Monilia found by culture				
						H B	Liv	Spl	Kid	P G
59	186 GP	A VIII	Sprue faeces	64	Not noted	—			—	—
60	187 GP	A VIII passed through GP	Do	2	Acute peritonitis	—			—	—
61	189 GP	A 29 passed through GP	Do	57	Not noted	—			—	—
62	190 GP	Do	Do	57	Do	—			—	—
63	227 GP	Blastomyces 43	Do	17	Plastic peritonitis	—			—	—
64	304 GP	A VIII	Sprue Human faeces	2	Acute peritonitis	—		+	+	+
65	317 WM	M CCXVII	Do	3	Nil	—			+	+
66	315 WM	A CVIII	Do	3	Plastic peritonitis	—				+
67	319 WM	A CXXIX	Do	3	Peritonitis	—				+
68	320 WM	A CXLIII	Dysentery	3	Localized peritonitis	—				+
69	321 WM	K 698 (L I T C)	L I P M	3	Acute peritonitis	—		+		+
70	322 WM	A CCLVIII	Sprue faeces	3	Localized peritonitis	—				+
71	323 WM	A VIII	Do	3	Elastic peritonitis	—		+	+	+
72	324 WM	A 1062 L I T C	L I P M	3	Do	—				+
73	325 WM	Albicans Crank L I T C 714	Do	3	Do	—				—
74	326 WM	A 29 Ash	Sprue faeces	3	Do	—				+
75	347 GP	A Gomes monilia	Duodenal fluid, sprue	25	Localized peritonitis	—			—	—
76	348 GP	Do	Do	30	Adhesions P O	—			—	—

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## PROTOCOL II

*Animals which received multiple intraperitoneal injections*

## (1) Animals which died after the injections

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Dates of inoculation	Date of death and P M signs	Monilia found by culture				
						H B	Lav	Spl	Kid	P C
1	125 GP	A 29	Sprue faeces	19-11-23 24-11-23 27-11-23 30-11-23 5-12-23	Died on 5-12-23 Plastic peritonitis	—				+
2	126 GP	A 30	Sprue tongue	19-11-23 24-11-23	Found dead 25-11-23 Plastic peritonitis Peri- cardial effusion			+		+
3	129 GP	A 30 passed through GP	Do	27-11-23 30-11-23 5-12-23 10-12-23	Died 10-12-23 Plastic peritonitis	+		+		+
4	219 GP	A 29	Sprue faeces	21- 7-24 23- 7-24 25- 7-24 30- 7-24	Found dead 31-7-24 Plastic peritonitis					
5	221 GP	A XXXIV	Faeces (disease not known)	21- 7-24 23- 7-24 25- 7-24 31- 7-24 1- 8-24	Died 6-8-24 Plastic peritonitis	—				



PROTOCOL II

(3) Animals which recovered and were returned to stock

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Dates of inoculation	REMARKS
10	210 R	K LITC 698	L I P V	8-7-24 15-7-24 22-7-24	Remained all right for 5 months
11	211 R	A 29	Sprue foci	8-7-24 15-7-24 22-7-24	
12	212 R	A 29	Do	8-7-24 15-7-24 22-7-24	
13	213 R	A VIII	Do	8-7-24 15-7-24 22-7-24	
14	217 R	K LITC 698	L I P V	15-7-24 22-7-24	

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## PROTOCOL IV.

*Animals which received multiple intravenous injections*

## (1) Animals which died after the injections

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Dates of inoculation	Date of death and P M signs	Monilia found by culture			
						H B	Liv	Spl	Kid P O
1	192 R	K L I T C 698	L I P M	14-5-24 15-5-24 16-5-24	Died 16-5-24 Nil				
2	197 R	A VIII	Sprue faeces	6-6-24 7-6-24 9-6-24	Died 10-6-24 Nil	+			+
3	198 R	A VIII	Do	6-6-24 7-6-24 9-6-24	Died 10-6-24 Necrotic specks on liv gall-bladder and wall of intestines None on kidneys	-			+
4	203 R	Albicans crak L I T C 714	L I P M	6-6-24 7-6-24 9-6-24	Died 14-6-24 Necrotic specks on kidneys	+			+
5	204 R	Do	Do	10-6-24 6-6-24 7-6-24	Died 10-6-24 Necrotic specks on wall of caecum	+			+

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## PROTOCOL IV

*Animals which received multiple intravenous injections—(contd.)*

(2) Animals which were killed after the injections—(contd.)

Serial No.	Animal number	Type and No of monilia strain	Source of virus	Dates of inoculation	Date of killing P M signs	Monilia found by cultures				
						H B	Lvs	Spl	Kid	P C
6	200 R	K LITC 698	LIPM	6-6-24 7-6-24 9-6-24 10-6-24	Killed 17-6-24 Necrotic specks on cecum					
7	201 R	K XIX	Chronic diarrhoea faeces	6-6-24 7-6-24 9-6-24 10-6-24	Killed 17-6-24 Nil				—	
8	202 P	Do	Do	6-6-24 7-6-24 9-6-24 10-6-24	Killed 17-6-24 Nil				—	
9	305 R	M CCXVII	Sprue faeces	8-7-27 11 7-27 25-7-27	Killed 3-8-27 Nil	—			—	
10	306 R	A CVIII	Do	8-7-27 11-7-27 25-7-27	Killed 3-8-27 White patches on kidney	—			—	
11	307 R	A CXXIX	Do	8-7-27 12-7-27 26-7-27	Killed 6-8-27 Nil	—			—	
12	308 R	A CXLIII	Dysentery faeces	8-7-27 11-7-27	Killed 25-7-27 Infarcts kidney	—		—	—	
13	390 R	K LITC 698	LIPM	8-7-27 11-7-27 26-7-27	Killed 6-8-27 Nil	—			—	

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## PROTOCOL V.

*Animals which were given by mouth (fed on) a single dose of Monilia Ashtfordi isolated from duodenal fluid of Gomes*

Serial No	Animal number.	Signs during life	When killed and P M signs	Monilia found by culture				
				H B	Liv	Spl	Kid	P C
1	361 R	Nil, motions solid	Killed on 24th day, nil	—				
2	362 R	Do	Do	—				
3	363 WM	Do	Killed on 23rd day, nil	—			—	
4	364 WM	Do	Do	—			—	
5	365 GP	Do	Do	—			—	
6	366 GP	Do	Do	—			—	







The dietary given to induce scurvy was that recommended by Lieutenant-Colonel R McCarrison, *RMS*, and consisted of—

Polished rice (boiled)	100 grms
Ground-nuts (parched)	30 grms
Autoclaved milk	200 c cs
Butter	10 grms
Orange juice	2 c cs

The infective feeds of sprue material were generally given when the animals began to lose weight and to exhibit symptoms of ill health. Where the scorbutic condition was advancing too rapidly, an attempt was made to check it by giving a small amount of orange juice or, in one or two cases, by putting the animal back on a full normal diet. In two animals so treated the signs of dysentery receded and the animals quickly recovered (Monkeys 279 and 280), but in monkeys in which a more marked scorbutic condition was present the exhibition of vitamin C was too late to save them.

The most marked result was a loss of weight. This was generally slight at first and was represented by a slow but steady decline in the earlier weeks. When once the decline was established, there came a point when the weight came tumbling down especially with the onset of dysentery and the loss increased in rapidity till the animal died. In some animals no definite signs of scurvy appeared or were found after death, but in others definite scorbutic signs appeared, such as puffiness and ecchymosis around the eyelids or other parts of the face. Small hæmorrhages or bruises under the skin and sometimes hæmorrhages into the muscles were found post-mortem. Loosening of the teeth and bleeding from the gums were seen in the more severe cases. Apathy, wasting and 'staring' of the coat were common signs, but diarrhœa was not a prominent feature during life except in a few cases.

The infected feeds were generally commenced when the animals' weight began to decline and consisted of saline suspensions of the fresh fæces of human sprue cases or of cultures of *Monilia psilosis* isolated therefrom or of both together.

#### *Post-mortem appearances*

On opening the abdomen, the intestine was generally seen to be injected and the large bowel was often thickened and congested. No obvious naked eye changes were seen in the viscera, but the heart was generally pale and flabby.

On opening up the alimentary canal, small hæmorrhages or diffuse ecchymosis were sometimes present in the stomach. The small intestines were frequently congested, but rarely showed much change till the lower part of the ileum was reached.

At this level the mucous membrane was thickened and velvety, whilst small superficial erosions were occasionally present, but ulceration was never seen above the level of the ileocæcal valve.

The large intestine showed well-marked changes in every case, particularly in the cæcum and the descending colon and sigmoid. The changes varied from

PROTOCOL I  
*Animals which received a single intraperitoneal injection*  
 (2) Animals which were killed after the injection

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Killed (days)	P M signs	Monilia found by culture			
						H B	Lav	Spl	Kid
5	78 R	A I	Sprue faeces	75	Nil	-		-	
6	79 GP	A I	Do	75	Nil	-		-	
7	81 WM	A I	Do	75	Nil	-		-	
8	82 WM	A I	Do	75	Nil	-		-	
9	84 GP	A I	Do	3	Acute peritonitis		-	+	+
10	85 GP	A I	Do	14	Nil	-	-	+	
11	86 GP	K III	Do	3	Peritonitis	-	+	+	
12	87 GP	An IV	Do	14	Nil		-		
13	88 GP	K V	Do	13	Nil	-		-	
14	89 GP	An VI	Do	3	Peritonitis	+	-	+	
15	90 GP	K VII	Do	14	Localized peritonitis		-		+
16	91 GP	A VIII	Do	3	Acute peritonitis		+		+
17	92 GP	An IX	Non sprue faeces	14	Nil	-		-	
18	95 GP	K XII	Normal faeces	14	Nil	-		-	
19	96 GP	K XIII	Do	14	Nil	-		-	
20	97 GP	An XIV	Dysentery	14	Nil	-		-	
21	98 GP	A XV	Diarrhoea	14	Encapsuled abscess P C	-		-	



disease (Fuller details will be found in the report on each monkey in the attached protocols)

#### REMARKS

On going through such literature as we have at our disposal, we find that though a great deal of work has been done on experimental scurvy in animals (including monkeys), references to lesions of the intestinal tract are very infrequent and it is clear that the extensive dysentery-like condition present in our monkeys is somewhat exceptional

Thus Harden and Silva (1918-1919) describe in detail the symptomatology of experimental scurvy in monkeys but make no reference to any intestinal lesions

On the subject of vitamins and infection, Findlay (1922) had made a series of observations on guinea-pigs suffering from experimental scurvy. No mention is made of the condition of the alimentary tract. He confirms the question of lowered resistance to bacterial infection. The result of his experiment with four species of bacteria shows that guinea-pigs, fed on a diet deficient in vitamin C, succumb to a smaller infecting dose of bacteria than animals fed on a complete diet and that the symptoms of toxæmia are manifested more rapidly in scorbutic than in control pigs. He associates this fact with an altered condition of the bone marrow and concludes that guinea-pigs with chronic scurvy, though showing few clinical symptoms, are less resistant to bacterial infection.

Several writers, Cohen and Mendel (1918), Jackson and Moody (1916), point out the lowered resistance to bacterial invasion which is seen in scorbutic animals, and this suggests that the dysentery-like condition found in our animals may have been due to the invasion of faecal organisms of exalted virulence rather than to specific organisms of human dysentery. The occasional absence of all clinical signs of dysentery during life in spite of the ulcerated condition present in the large intestine as revealed by necropsy is a remarkable clinical fact.

It recalls an incident which the senior writer witnessed in Mesopotamia during the late war. An officer had been invalided from the front after suffering considerable hardship, including poor feeding, and was admitted to the officers' hospital in Baghdad for some minor surgical condition. He was under the close observation of experienced surgeons and trained nurses for a week after admission but at no time presented any signs of intestinal disease and his motions were reported to be normal. He died suddenly of heart failure (in itself a suspicious sign of avitaminosis) and at the autopsy his large intestine was found to be in a condition resembling acute dysentery with extensive superficial ulceration and sloughing of the mucous membrane. Scurvy was at that time rife in the outposts of the war area and one is tempted to compare the condition in this patient with what one observed in several of the monkeys in the present experiments. About the same time a terrific outbreak of dysentery occurred amongst a number of refugees who had recently arrived in Baghdad in a condition of extreme malnutrition and poverty, being a survival of those who escaped massacre at the hands of the Turks in the highlands of Persia. The appalling severity of the epidemic (resembling cholera at its worst) and the profound intestinal changes

[illegible]

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PROTOCOL I  
*Animals which received a single intraperitoneal injection*  
 (3) Animals returned to stock after the injection

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Results
77	114 GP	K XXXI	Dysentery	Recovered without symptoms
78	115 GP	Aa XXXII	Do	
79	116 GP	Kk XXIX	Sprue faeces	
80	117 GP	Kk XXX	Do	
81	118 GP	M XXXIII	Diarrhoea	
82	119 GP	A XXXIV	Faeces (disease not known)	
83	131 GP	Aa XXXVI	Diarrhoea	
84	132 GP	Aa XXXVII	Do	
85	134 GP	A 30	Tongue sprue	
86	136 GP	A LIV	Diarrhoea	
87	75 Mk	A I	Sprue faeces	Do
88	231 GP	A CX	Healthy faeces	Do
89	232 GP	A <sub>1</sub> CXII	Do	Do
90	233 GP	A CXIV	Do	Do
91	234 GP	Aa CXV	Do	Do

Monilia were not found to have invaded the diseased areas nor can the changes be attributed to their action

8 A defective dietary by itself acts as a powerful predisposing cause of bowel derangements and acts probably by reducing the natural resistance of the intestinal epithelium to the invasion of bacteria or their toxins

## REFERENCES

- |                              |   |
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| MCCARRISON (1918)            | The Pathogenesis of Deficiency Disease <i>Ind Jour Med Res</i> , Vol VI, Jan, p 344   |
| MCCARRISON (1919)            | The Pathogenesis of Deficiency Disease (1) The Effect of Autocalved Rice Dietaries on the Gastro-intestinal Tract of Monkeys <i>Ind Jour Med Res</i> , Vol VII, No 2, Oct, p 283, (2) The General Effects of Deficient Dietaries on Monkeys <i>Ibid</i> , Vol VIII, p 308 |

## Protocol II

## (2) Animals which were killed after the injections

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Dates of inoculation	Date of death and P M. signs	Monilia found by culture			
						H B	Liv	Spl.	Kid P C
6	218 GP	A 29	Sprue faeces	21-7-24 23-7-24 25-7-24 30-7-24 1-8-24	Killed 28-8-24 Adhesions P C				
7	220 GP	A XXXIV	Faeces	21-7-24 23-7-24 25-7-24 30-7-24 1-8-24	Killed 28-8-24 Plastic peritonitis				
8	222 GP	K LITC 608	LIPM	21-7-24 23-7-24 25-7-24 30-7-24 1-8-24	Killed 28-8-24 Plastic peritonitis				
9	223 GP	Do	Do	21-7-24 23-7-24 25-7-24 30-7-24 1-8-24	Killed 28-8-24 Plastic peritonitis				

*Monkey 279*

Put on deficiency diet on 25th February 1927

Infected material given —

29th March 1927, saline suspension of sprue faeces

2nd April 1927, culture from sprue faeces

8th April 1927, saline suspension of sprue faeces

19th May 1927     "             "             "             "

11th June 1927     "             "             "             "

*Clinical Course*—Weight fell gradually from 2,000 to 1,850 grammes between commencement of experiment to middle of May. Thence dropped rapidly to 1,400 grammes about 20th June. Then began to rise and reached 1,850 in mid-September. Showed signs of dysentery on 21st June 1927. Continued ill for ten days showing blood-stained mucus and made gradual recovery after having been put back on full diet on 26th June 1927. Blood examinations at the height of dysentery showed severe anæmia

R B C	1,375,000
W B C	12,500
Hb	50 per cent
H I	18

No nucleated reds

*Bacteriology of Faeces*—Fresh preparations showed trichomonads and their cysts, yeasts and their mycelia, a few bodies resembling coccidia. No amoebæ. Bacterial flora appeared normal.

Previous to commencement of experiment, monilia of *psilosis* type and of M type (cryptococcus) were found and subsequent to giving infected material *M. psilosis* and cryptococci were again found.

Bacteriology of faeces was not done.

*Result*—The animal developed dysentery but recovered on being placed on a diet abundant in vitamin C.

*Monkey 280*

Deficiency diet begun on 25th February 1927

Fæcal examination previous to giving infected material, monilia of species undetermined found on one occasion.

Infected feeds —

29th March 1927, saline suspension sprue faeces

2nd April 1927, culture from sprue faeces

8th April 1927, saline suspension sprue faeces

19th May 1927     "             "             "             "

11th June 1927     "             "             "             "

*Clinical Course*—Showed gradual decline in weight from 1,630 to 1,480 grammes and a sudden drop about the middle of May to 1,240 grammes. At the end of May, began to rise in weight and eventually approached 1,600 grammes.

## PROTOCOL III

*Animals which received a single intravenous injection*

## (1) Animals which died after the injection

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Died (days)	P M signs	Monilia found by culture				
						H B	Liv	Spl	Kid	P O
1	344 R	A Gome/ monilia	Duodenal fluid sprue	2	Nil, except pleural and pericardial effusion	+	+	+	+	+
2	345 R	Do	Do	3	Necrotic specks, myocardium, liv, kid and appendix	+	+	+	+	+
3	346 R	Do	Do	2	Necrotic specks on appendix, pleural and pericardial effusion	+	+	+	+	+
4	369 P	Do	Do	6	Necrotic specks on liver and kidneys	—			+	

## (2) Animals which were killed after the injection

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Killed (days)	P M signs	Monilia found by culture				
						H B	Liv	Spl	Kid	P O
5	103 R	A VIII	Sprue faeces	38	Nil	—		—		
6	104 R	Aa VI	Do	38	Nil	—		—		
7	250 R	43 Blastomyces	Anstralia	17	Nil	—			—	
8	349 R	A Gome/ monilia	Duodenal fluid sprue	3	Nil, except necrotic specks on myocardium and kidneys		—	+	+	+
9	350 R	Do	Do	4	Nil, except pleural effusion and necrotic specks on kidneys	—	—	—	+	+
10	370 R	Do	Do	4	Necrotic specks on kidneys	—			+	+

## (3) Animals which recovered and were returned to stock after the injection

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Returned (days)	Result
11	76 Mk	A I	Sprue faeces		Recovered without symptoms
12	77 R	A I	Do		Do



inflamed throughout and covered with mucus. No ulcers were seen, but the gut was thickened and the submucous and mucous coats were in a state of subacute inflammation.

*Micro-histology*—The glands at pyloric end of the stomach were markedly degenerated almost resembling amyloid substance but there were no signs of inflammation. The cæcum showed extensive degeneration of surface epithelium without inflammatory reaction. The prevailing organisms were large curved beaded Gram-positive bacteria resembling those showing serpiginous motions seen in fresh preparation. The signs in the large intestine were less marked but there was some superficial necrosis of mucous membrane. Liver, kidney and spleen showed no obvious changes.

### *Monkey 282*

Deficiency diet begun on 25th February 1927

Fæcal examinations showed no moulia

Infected feeds —

25th April 1927, culture of *M. psilosis* (Ashford) (previously passed through guinea-pig)

21st May 1927, culture of another strain of *M. psilosis* also from sprue case

*Clinical Course*—Began to lose weight soon after the first infected feed and dropped rapidly from 1,600 to 1,040 grammes during a period of seven weeks, after which it was killed on account of its advanced condition of debility. Developed diarrhœa on 19th May 1927. Motions became dysenteric on 11th June 1927, consisting of blood-tinged mucus, with loose green and blood-stained fæces. Monkey put on ordinary diet on 13th June 1927 continued to lose weight and the dysentery continued. The blood was examined on three occasions after the onset of diarrhœa, but did not show any abnormal cytology.

*Fæcal Examination*—13th June 1927. Flagellates present and a number of large slowly undulating bacilli with sinuous outline. *M. psilosis* and *M. krusci* were recovered from the fæces. Bacteriological examination showed only cocci and coliform bacilli. No lactose non-fermenters were found and the large bacilli above referred to were not found on the (aerobic) plates.

*Post-mortem*—Killed on 15th June 1927 in a very emaciated condition (weight 1,040 grammes). The large intestine was distended and thickened, the mucous membrane was velvety and much thickened as was also the submucous layer. Some hæmorrhagic areas were noted but no actual ulceration. The small intestine presented no naked eye changes. There was some blood-stained fluid in the peritoneal cavity but no pus or adhesions. The bone marrow appeared normal.

*Micro-histology*—The stomach and duodenum were normal. The cæcum and ascending colon showed denudation of epithelium in some areas and superficial erosion not amounting to actual ulceration. Some superficial small celled and lymphoid exudation in submucosa but very little sign of inflammation or congestion, but more those of toxic or degenerative changes.

## PROTOCOL IV

*Animals which received multiple intravenous injections*  
 (2) Animals which were killed after the injections

Serial No	Animal number	Type and No of monilia strain	Source of yeast	Dates of inoculation	Date of killing P M signs	Monilia found by culture				
						H B	L v	Spl	Kid	P C
1	83 R	A 1	Sprue faeces	9-7-23 11-7-23 13-7-23 16-7-23 17-7-23 19-7-23 21-7-23	Killed 14-8-23 Nil	—	—	—	—	—
2	104 R	A 30	Sprue tongue	14-5-24 16-5-24 16-5-24 17-5-24	Killed 19-5-24 Necrotic specks kidneys and peritoneal effusion	—	—	—	+	—
3	105 R	A 20	Sprue faeces	6-6-24 7-6-24 9-6-24 10-6-24	Killed 11-6-24 Necrotic specks kidney, caecum and gall bladder	+	—	—	+	—
4	106 R	A 20	Do	6-6-24 7-6-24 9-6-24 10-6-24	Killed 17-6-24 Necrotic specks kidney and caecum	—	—	—	+	—
5	109 R	K LITC 698	L I P M	6-6-24 7-6-24 9-6-24 10-6-24	Killed 17-6-24 P M signs not recorded	—	—	—	—	—

*Clinical Course*—The maximum weight of the animal, 3,370 grammes, was noted at the beginning of October, after which it fell almost vertically till 26th November 1927, when it weighed 2,190 grammes and was killed in a state of extreme emaciation. Commenced to show signs of scurvy on 8th October 1927, eyelids swollen and ecchymosis of the face, gums scorbutic and bleeding, conjunctiva inflamed, later some teeth fell out and signs of scurvy increased. No signs of dysentery or diarrhoea during life.

*Bacteriological Examination*—The faeces contained a coliform Gram-negative bacillus, forming acid in lactose and acid and gas in glucose, maltose, saccharose and mannite, presumably one of the *B. coli* group. Numerous Gram-positive cocci giving acid in glucose, lactose, maltose, saccharose and mannite, acid and clot in milk. No lactose non-fermenters were found. *M. psilosis* and streptococci recovered from the intestinal contents.

*Post-mortem*—The large intestine and especially the caecum was inflamed and the mucous membrane thickened and velvety. The walls were injected and thickened but no ulceration was seen. The condition of the ileum was similar, but in the small bowel there was only thickening of the mucous membrane without denudation. The other organs showed no change.

*Micro-histology*—The alimentary canal down to the lower end of the ileum showed little alteration, but at that level there was desquamation of surface epithelium and coagulation necrosis of these structures. The large intestine was more extensively diseased and the mucosa was affected as far down as the submucous and muscular layer. There was loss of staining reaction, a condition of lymphoid hyperplasia and well-marked congestion. Blood was extravasated widely into the mucous and submucous layers and the muscular layers were in a state of cloudy swelling or early toxic necrosis. The liver and kidney showed marked toxic changes. The liver showed abundance of haemosiderin.

### *Monkey 328*

Deficiency diet begun on 8th August 1927

Infected feeds —

12th September 1927, saline suspension of sprue faeces (Gomez)

17th October 1927       "               "               "               "

*Clinical Course*—The animal kept its weight fairly well, 3,600 to 3,400 grammes, for about three months and then rapidly dropped to about 3,200 grammes in the last two weeks of life. General condition was fairly good till just before death and the animal showed no evidence of scurvy and no signs of diarrhoea or dysentery during life. It died unexpectedly on 21st November 1927.

*Bacteriological Examination*—No amœbæ or other protozoa seen. The large undulating motile bacillus previously noted was abundant. The usual cocci and coliform bacilli were found on culture and otherwise the predominant bacillus was a thin bacillus, Gram-negative and non-motile, which fermented glucose, maltose and saccharose, acid without clot in milk, and produced indol. It did not ferment lactose, mannite or dulcitol. The large serpiginous bacillus was not isolated.

14	310 R	A CGLVIII	Sprue faeces	8-7-27 11-7-27 25-7-27	Killed 8-8-27 Necrotic specks and infarcts kidney	-	-	-
15	311 R	A VIII	Do	8-7-27 11-7-27 25-7-27	Killed 8-8-27 Necrotic specks and infarcts kidney	-	-	-
16	312 R	A LITC 1062	LIPM	8-7-27 11-7-27 26-7-27	Killed 10-8-27 Necrotic specks and infarcts kidney	-	-	-
17	313 R	Albicans crank LITC 714	Do	8-7-27 11-7-27 25-7-27	Killed 10-8-27 Minute healed scar kidney	-	-	-
18	314 R	A 29	Sprue faeces	8-7-27 11-7-27 25-7-27	Killed 10-8-27 Necrotic specks and linear radiating infarcts kidney	-	-	-
19	333 R	A 29 passed through mouse	Do	12-8-27 15-8-27 18-8-27	Killed 13-9-27 Necrotic foci kidney	-	-	-
20	335 R	A CXLIII passed through mouse	Dysentery faeces	12-8-27 15-8-27 18-8-27	Killed 5-9-27 Infarcts kidney	-	-	-
21	336 R	LITC K 698 passed through mouse	LIPM	12-8-27 15-8-27 19-8-27	Killed 5-9-27 Nil	-	-	-
22	337 R	Albicans crank LITC 714	Do	12-8-27 15-8-27 18-8-27	Killed 5-9-27 Nil	-	-	-

## CONTROL MONKEYS 330, 331 AND 332

Kept on deficiency diet but given no infected feeds

*Control Monkey 330*

Deficiency diet begun on 8th August 1927

*Clinical Course*—Maximum weight 2,950 grammes noted about two months after commencement of experiment. Fell gradually till mid-November, when it was recorded as 2,870 grammes. After this the weight dropped rapidly and was 2,150 grammes at death. Showed some loosening of teeth about 14th October 1927, but no other signs of scurvy and it remained active till 30th November 1927, when it suddenly fell ill, developed dysentery and died on 1st December 1927.

*Bacteriological Examination*—The dysenteric faeces, both before and after death, were examined but showed no amoebæ or cysts. Some flagellates and spirochaetes were present. On culture bacilli with reaction of *B. coli* were isolated from the heart blood, but no abnormal bacteria were found on faecal culture.

*Post-mortem*—The alimentary canal down to the caecum showed no change and the principal viscera also appeared normal. The caecum and adjoining part of large intestine was deeply injected and inflamed and resembled acute dysentery. The mucous membrane was congested, thickened and velvety and showed well-marked ulceration in patches. There was an ulcer also in the sigmoid flexure. Small hæmorrhages were scattered through the large bowel.

*Micro-histology*—The changes in the alimentary tract were similar but not so advanced as in Monkey 328. The small intestine showed decided degenerative changes with withering of the villi and superficial desquamation. The mesenteric glands showed well-marked toxic necrosis and the spleen contained a large amount of black pigment which did not give the iron reaction. The liver and spleen showed toxic changes in the parenchyma. The large bowel was extensively diseased as in the previous cases.

*Control Monkey 331*

Deficiency diet commenced on 8th August 1927

No infected feeds

*Clinical Course*—Its weight, about 3,000 grammes, was maintained till the end of October, when it commenced to fall rapidly till it reached 2,080 grammes at the time of death on 28th November 1927. Signs of scurvy were noted on 29th October 1927 and were noted irregularly till time of death. Diarrhoea noted on 26th November 1927. Dysenteric symptoms not noted. The animal became very ill a few days before death.

*Bacteriological Examination*—No amoebæ found in fresh preparations. The plates showed the usual growth of cocci and coliform lactose fermenters, but there were also present a number of lactose non-fermenters which gave the reaction of *B. dysenteriae* Flexner and were agglutinated by both 'Flexner and Y' sera. This appeared to be a genuine 'Flexner and Y' dysentery.

*Post-mortem*—The mucous membrane of the stomach was congested and showed ecchymosis in several places. The small intestine showed no change

## PROTOCOL VI

*Animals inoculated with duodenal fluid of sprue patients or with organisms isolated from it*

Serial No	Animal number	Material inoculated	Date and how inoculated	Result
1	290 GP	Duodenal fluid	I P 10-3-27	Returned to stock having remained all right up to 6-4-27
2	291 GP	Do	S C 10-3-27	Died on 14-3-27 P M—Pericardial effusion Lungs consolidated Muscular and subcutaneous congestion Gram negative coliform bacilli in H B spleen, liver and lungs
3	292 GP	Do	S C 11-3-27	Killed on 6-4-27 P M—Nil, except abscess at seat of inoculation
4	293 GP	Do	Do	Returned to stock on 6-4-27 having remained all right except for local abscess
5	294 GP	Do	I P 11-3-27	Returned to stock having remained all right up to 8-4-27
6	295 GP	Do	Do	Do
7	296 GP	Do	S C 16-3-27	Died 5-4-27 P M—Nil, except mild peritonitis and slough at seat of inoculation, latter showing +++ bacilli and cocci
8	297 GP	Do	Do	Remained all right except for abscess at the seat of inoculation Returned to stock on 6-4-27
9	298 GP	Culture (Gram + diplococci)	S C 22-3-27	Remained all right and returned to stock on 14-4-27
10	299 GP	Culture sporing stout bacillus	Do	Do
11	300 GP	Culture (Gram + thin bacillus)	Do	Do

## PROTOCOL II

*Characters of different types of bacilli and cocci isolated from the contents of intestine of seven of the experimental monkeys*

Serial Numbers	Morphology	SUGAR FERMENTATION REACTIONS											REMARKS
		Gram	Motility	Lactose	Glucose	Maltose	Saccharose	Mannite	Dulcite	Litmus milk	Indol	Hæmolytic power	
I <i>Lactose non fermenters</i>													
1	B (coliform)	—	—	—	A	—	A	—	—	+	—	Flexner type	
2	B ( „ )	—	—	—	AG	AG	AG	—	—	A	—		
3	B ( „ )	—	—	—	AG	AG	—	AG	—	—	—		
4	B (fusiform sporing)	—	—	—	A	A	—	—	—	—	—		
5	B (short)	—	+	—	A	—	—	—	—	—	—		
II <i>Lactose fermenters</i>													
6	B (coliform)	—	—	A	A	A	A	A	A	—	—	—	
7	B (short, thin)	—	—	A	AG	AG	AG	AG	—	AG	—		
8	B ( „ „ )	—	—	A	A	—	A	A	—	A	—		
9	B ( „ „ )	—	—	AG	AG	AG	—	AG	AG	AG	—		
10	B (coliform)	—	—	AG	AG	AG	—	AG	AG	AG	—		
11	B ( „ )	—	—	AG	AG	AG	—	AG	AG	A	+	—	
12	B ( „ )	—	+	AG	AG	AG	AG	AG	—	A	—	—	
13	B (medium thickness)	—	—	AG	AG	AG	—	AG	—	A	+	—	
14	B (coliform)	—	—	AG	AG	AG	AG	AG	—	C	—	—	
15	B ( „ )	—	—	—	—	—	—	—	—	A	—	—	
III <i>Cocci</i>													
16	Co (round)	+	—	—	—	—	—	—	—	—	—	—	
17	Co (minute diplo)	+	—	—	A	—	—	—	—	—	—	—	
18	Co (diplo)	+	—	—	A	—	A	—	—	C	—	—	
19	Co (large)	+	—	—	A	A	A	A	A	—	—	—	
20	Co ( „ )	+	—	—	A	A	A	—	—	—	—	—	
21	Co	+	—	A	A	A	A	—	—	A	—	—	
22	Co (small, round)	+	—	A	A	A	A	A	—	A	—	—	
23	Co (minute)	+	—	A	A	A	A	A	—	C	—	—	
24	Co (diplo)	+	—	—	A	A	A	—	—	—	—	—	

Note—Group I Contains lactose non fermenters

Group II. Contains lactose fermenters mostly *B coli* and its congeners

Group III Contains the various types of cocci present

# THE ASSOCIATION OF BOWEL DISEASE WITH VITAMIN C DEFICIENCY

BY

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(Being Part III of the Sprue Investigation at the Haffkine Institute, Parel,  
Bombay)

[Received for publication, February 27, 1928]

We attempted in a series of experiments detailed in a previous paper\* to produce the clinical feature of sprue in animals by infecting them with sprue faeces or with monilia isolated therefrom. In some of these experiments we tried to induce susceptibility in monkeys by keeping them on a diet deficient in vitamin C. We were not successful in reproducing sprue in these animals, but we found that avitaminosis in itself produced an increased susceptibility to disease of the intestinal tract.

We used sixteen monkeys (*M. sinicus* and *rhesus*) in two batches. The first batch included ten monkeys including four controls which latter were fed on a normal diet and which did not receive infected material. In this batch the six monkeys fed on a deficient diet which also received infective feeds of sprue material, all developed an inflammatory or degenerative condition of the alimentary tract resembling dysentery, whilst the four control monkeys remained quite free from this disease.

This batch of experiments suggested that the feeds of infective material from sprue cases were responsible for the bowel disease referred to.

The second batch of experiments was more exacting in that all the six monkeys were maintained on a deficient diet, but the three controls received no infective feeds. The six animals were housed on the same verandah but were separate from each other. All six animals developed the same clinical symptoms and showed the same post-mortem conditions, so it is fair to assume that the causative or predisposing factor was the deficiency in vitamin C and not the infected feeds.

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\* 'Animal Experiments and Sprue' (*this number*), pp 49—75





Fig 1

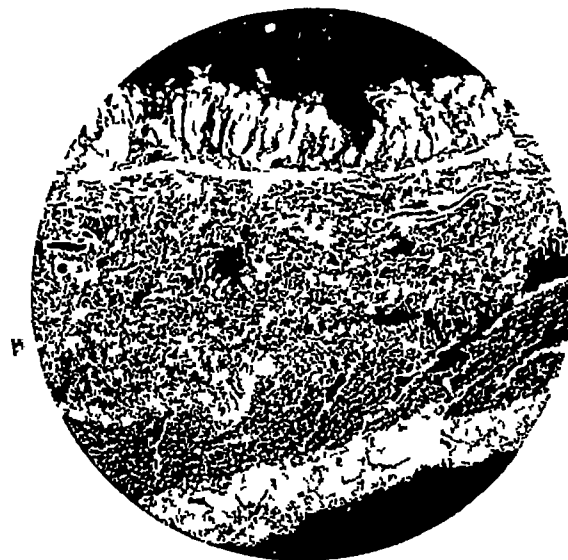


Fig 2



Fig 3



Fig 4



deep congestion with thickening of the mucous and submucous layers to frank ulceration and widespread superficial sloughing of the mucous membrane. Perforation of the cæcum and invasion of the adjoining surface of the liver was noted in one case. The condition in the severe cases resembled a toxic form of dysentery with ulcers, hæmorrhages and surface sloughs. The impression one gained was that the inflammatory changes were less obvious than those of degeneration and this was borne out by a study of the micro-histology.

#### *Micro-histology.*

The stomach and upper part of the small intestine rarely showed any change except for small hæmorrhages which were part of the scorbutic state. The lower portion of the ileum was generally congested and the mucous membrane thickened. In places there was loss of staining power and cloudy swelling of the superficial layers suggesting an early stage of toxæmia. The large intestine in every instance showed these inflammatory and toxic changes more profoundly. Wide areas were almost denuded of the surface epithelium which lay in the lumen in a state of coagulative necrosis containing swarms of bacteria. Ulceration was seen in some specimens extending to the submucosa or more rarely to the muscular coat. In the more profound cases the whole of the gut wall was swollen and the muscle cells showed diffuse eosin staining with loss of nuclear definition as if the local toxins had affected these structures also.

The parenchyma of the liver, kidney and mesenteric glands often showed well-marked toxic changes as evidenced by obliteration of cell outlines, diffuse staining and small celled infiltration.

The blood of some of the animals was studied during life and revealed a progressive anaemia without signs of regeneration and with little change in the bone marrow after death. The intestinal mucosa was invaded by numerous bacteria chiefly cocci and Gram-negative coliforms which were mostly in the degenerated layers or in sloughs, but there was some evidence of deeper invasion of bacteria into the living tissues. Monilia were never seen to have invaded the living tissues, though they were often present in the faecal contents and in the sloughs.

#### *Bacteriological examination of the diseased intestine*

Examinations of the dysentery-like faeces both before life and immediately after death were carried out in nearly every case. Fresh scrapings from the large intestine never showed amœbæ or their cysts. Flagellates and blastocysts and bodies resembling coccidia were found on occasions. Large undulating slowly-motile bacteria were seen on several occasions but were not grown in any of the media used.

The faecal organisms were studied in most cases, but only on one occasion was a dysentery-like organism (of Flexner Y type) isolated and a proteus-like bacillus also in one or two occasions. Non-hæmolytic cocci were numerous in all cases. Monilia of *psilosis* type were sometimes isolated from the faeces before the experiment was begun and in most cases were recovered from the diarrhoeal or dysenteric stools, but there is no reason to associate them with the evolution of the bowel

### EXPLANATION OF PLATE XII

- Fig 1 Changes in duodenum of Monkey 332 similar to preceding
- Figs 2, 3 and 4 Cæcum of Monkey 328 showing the structure of one of the nodules depicted in the coloured plate Extensive coagulation necrosis of mucous membrane with fibrosis and small celled infiltration of submucous layers All glandular and epithelial elements are destroyed and replaced by subacute inflammatory tissue
- Fig 5 Low power section of liver showing toxic changes in lower cells and their destruction in certain areas
- „ 6 Same as Fig 5 under higher magnification showing toxic changes in liver cells

found at the post-mortem impressed one with the conviction that the condition of malnutrition was largely responsible for the virulence of the epidemic. The prevailing organism found was Shiga's bacillus, but the predisposing cause was the scorbutic or sub-scorbutic state which starvation had impressed on these unfortunate people.

The writer who has laid most stress on the intestinal changes in avitaminosis is McCarrison (1918) and he also has called attention to the deficient resistance to bacterial invasion which is brought about by a vitamin-deficient diet. His principal researches in this connection are described in two papers (McCarrison, 1919 and 1920) and an abstract of these papers is given in *Brit Med Jour*, February 21, 1920, p 249. A study of these researches reveals results with which the observations in the present paper are in close accord.

The symptoms and progress of the disease in monkeys as also the post-mortem signs and the morbid histology are almost precisely those which we have described, the only difference being that in McCarrison's series the duodenum and upper part of the small intestine were equally affected with the large intestine, whereas in our series the upper part of the alimentary canal was almost always free from disease. McCarrison appears to have satisfied himself of the existence of amoebic infection in some of his cases, whereas we were unable to find any such cause. He did not carry out bacteriological examinations of the intestinal contents so that the causation of the dysentery-like condition, if bacterial, was not demonstrated in his animals.

### CONCLUSIONS

1 Monkeys which are fed on a dietary deficient in or lacking 'vitamin C' become debilitated and anæmic, lose weight rapidly and generally suffer from a terminal dysentery which ends in death. Definite signs of scurvy appear in most of the animals.

2 This scorbutic condition can be checked by the administration of orange juice or by a return to a normal dietary provided it is not advanced. When once the scorbutic condition is well established, this treatment cannot be depended upon to save the animals' lives.

3 The post-mortem signs are most marked in the large intestine which shows a succession of changes suggestive of local poisoning. These signs vary from local congestion and thickening of the mucous membrane to a condition indistinguishable from ulcerating and sloughing dysentery.

4 The morbid histology suggests degenerative changes due to the action of a toxin to which inflammatory changes are added secondarily.

5 Specific excitants of dysentery such as amoebæ or dysentery bacilli are not found in the large majority of cases.

6 The commoner faecal flora are found in some cases to have invaded the living tissues of the bowel wall and this suggests that the toxic changes observed are the effect of such bacteria acting on tissues devitalised by the ill-balanced dietary.

7 These changes appeared in the alimentary canal in all animals kept on a diet deficient in vitamin C whether given infective sprue material or not.



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Fig. 1

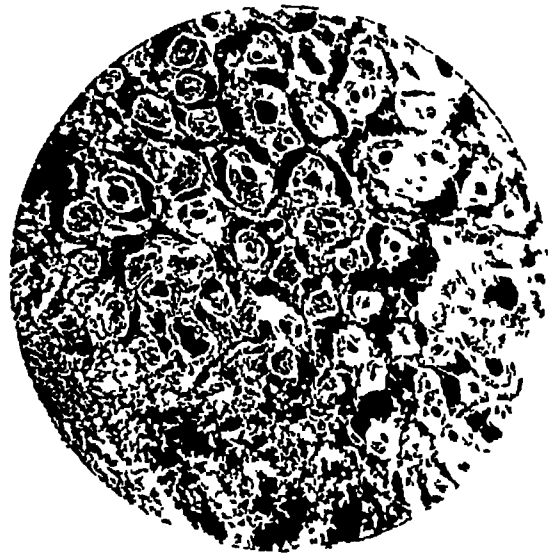


Fig. 2

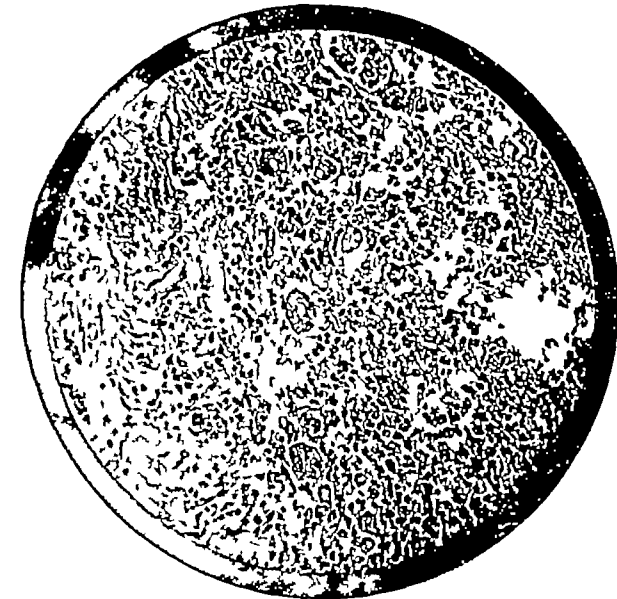


Fig. 3

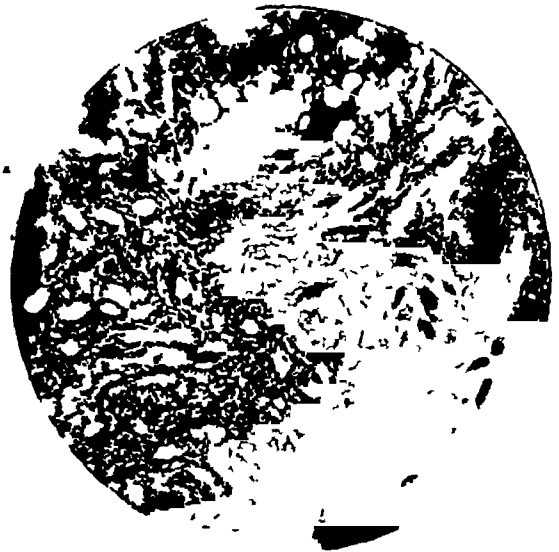


Fig. 4



Fig. 5



Fig. 6

## PROTOCOLS

## Protocol I

## Monkey 278

Put on deficiency diet on 25th February 1927

Infected feeds, 29th March 1927, saline suspension of sprue faeces

2nd April 1927, culture from sprue faeces

8th April 1927, saline suspension of sprue faeces

19th May 1927     "             "             "             "

11th June 1927   "             "             "             "

*Clinical Course*—Gradual loss of weight from 1,600 to 1,300 grammes (*vide* Charts I and II) No subcutaneous hæmorrhages or bleeding of gums noted Signs of dysentery set in on 20th June 1927 Faeces were normal till this date, but subsequently they became loose and contained blood and mucus Blood films examined during the course of the experiment showed no abnormality Monilia of 'M' type were found on two occasions in the faeces before the experiment was begun *M. psilosis* found on several occasions after commencement of infected feeds and also after death

Bacteriology of faeces after the onset of dysentery—

(1) Numerous strains of a Gram-positive coccus giving the following reactions—acid in lactose, glucose, maltose, saccharose, galactose and milk Mannite and dulcitate nil

(2) None of these strains were hæmolytic

(3) Strains giving the reaction of *B. coli* or *B. anærogenes*

(4) Strains giving the reactions resembling *B. paracolon*

(5) A strain resembling *B. faecalis alcaligenes*

*Post-mortem Signs*—Died 22nd June 1927

Stomach deeply injected and shows submucous hæmorrhages Small intestine deeply injected, mucous membrane velvety, no surface lesions

Large intestine acutely inflamed and shows the presence of small superficial ulcers and erosions

No naked eye changes in the other organs

Femur contains red marrow

No monilia found in heart's blood or viscera

Fresh scrapings from the inflamed gut showed trichomonads, yeasts and blastocysts but no amœbæ or cysts

*Micro-histology*—Stomach normal

Small intestine showed some aggregation of small cells under mucosa and epithelium was in a condition of cloudy swelling Large intestine showed early coagulative necrosis of superficial layers of epithelium with denudation and actual ulceration extending to submucous layer in places The muscular coat was swollen and blurred as if toxic Crowds of bacilli in necrosed areas Signs mostly toxic and little evidence of inflammation

Kidney epithelium showed toxic changes



#### EXPLANATION OF PLATE XIV

Shows the cæcum of experimental Monkey 328 The mucous membrane is inflamed and there are a number of necrotic nodules capped with sloughs protruding from the surface

Diarrhoea commenced on 26th May 1927, which continued off and on till the end of June. The faeces at their worst were thin and sanious-like meat juice. The monkey was put on ordinary diet on 26th May 1927, when the dysentery symptoms started, but the symptoms continued off and on for a month and then gradually cleared up as the animal improved in weight and in general health.

*Examination of Stool*—On 20th June 1927. Some flagellates, trichomonas and cysts. Yeasts. Bodies resembling coccidia. No amoebæ seen. Blastocysts present. The faeces were not examined bacteriologically. Monilia of types *M. psilosis*, *krusei* and 'M' cryptococcus were isolated from stools during the course of the dysentery.

*Remarks*—This animal began to show signs of dysentery and marked loss of weight two months after the first infected feed. It was then put back on full diet and the dysenteric signs gradually subsided and a month later the animal began to resume its normal health.

### Monkey 281

Put on deficiency diet on 25th February 1927

Faecal examination previous to infected feeds. *M. psilosis* and cryptococcus (M type) present

Infected feeds—

29th March 1927, saline suspension sprue faeces

2nd April 1927, culture from sprue faeces

8th April 1927, saline suspension sprue faeces

19th May 1927     "     "     "     "

11th June 1927   "     "     "     "

*Clinical Course*—The animal began to lose weight soon after the dietary was curtailed and dropped from 1,750 to 1,600 grammes by mid-May. After this dropped rapidly till date of its death on June 23rd, when it weighed 1,000 grammes.

Developed dysentery on 20th June 1927. Motions resembled liquid meat juice with blood-tinged mucus and the animal was very anæmic and the blood films showed anisocytosis, and poikilocytosis without nucleated red cells or other signs of regeneration.

*Examination of Stool*—20th June 1927. Flagellates and cysts with yeasts and mycelia, a few coccidia-like bodies. No amoebæ seen. Monilia of *psilosis*, *krusei* and *cryptococcus* types were isolated from the faeces. Gram-positive cocci giving reactions similar to those described under Monkey 278 were numerous. Those of the coli group were abundant. Several strains of a Gram-negative, non-motile aerobic bacillus of coliform type having the following sugar reactions—lactose and dulcitol negative, acid only in glucose, saccharose, mannite and milk. (The reactions in maltose and galactose were inconclusive.) These strains were not agglutinated by any of the high titre dysentery sera.

*Post-mortem Appearances*—Died on 24th June 1927. There were abundant submucous hæmorrhages near the pyloric end of the stomach. The small intestine was injected but showed no breach of surface. The large intestine was



*Monkey 283*

Deficiency diet begun on 25th February 1927

Fæcal examinations showed monilia of 'M' type (cryptococcus)

Infected feeds —

25th April 1927, culture of *M psilosis* from sprue patient (previously passed through guinea-pig)

21st May 1927, another strain of some monilia from sprue fæces

*Clinical Course* — The weight fell gradually from 1,640 grammes to 1,050 grammes at the autopsy. Dysentery commenced on 11th June 1927. Blood-tinged mucus with liquid or semi-solid greenish or blood-streaked fæces. The cytology of the blood remained normal. Put on ordinary diet on 13th June 1927, but did not recover and died on 17th June 1927.

*Fæcal Examination* — Fresh preparation showed nothing abnormal. Bacteriological examination showed the usual flora, and no organisms of dysentery group or other lactose non-fermenters were found. *M psilosis* was recovered from the fæces.

*Post-mortem on 17th June 1927* — The large intestine was in a condition of subacute dysentery with erosion of surface and small ulcers and a thick layer of blood-stained mucus. The small intestine was infected but showed no ulceration. The other organs including the bone marrow were normal.

*Micro-histology* — The whole of the large bowel from the cæcum to the rectum showed varying degrees of coagulation necrosis of whole depth of epithelium which in most areas had separated off or lay attached. The necrotic tissues swarmed with bacteria which penetrated to the submucous coat. Very little inflammatory reaction considering the profound changes seen in the mucous membrane. The mesenteric glands draining the affected area were intensely congested and hyperplastic and many large macrophages were present amongst the lymphoid tissue.

*Monkeys 284, 285, 286 and 287*

These were control monkeys fed on ordinary diet to whom no infected feeds were given. The first two kept their weight, remained healthy and were returned to stock. The other two died seven to ten months after commencement of observation of causes unknown (one probably caused by injury during handling). No signs of dysentery were found in either case.

*Monkey 327*

Deficiency diet begun on 8th August 1927

Infected feeds —

23rd August 1927, culture of *M psilosis* (Gomez)

29th August 1927       "       "       "

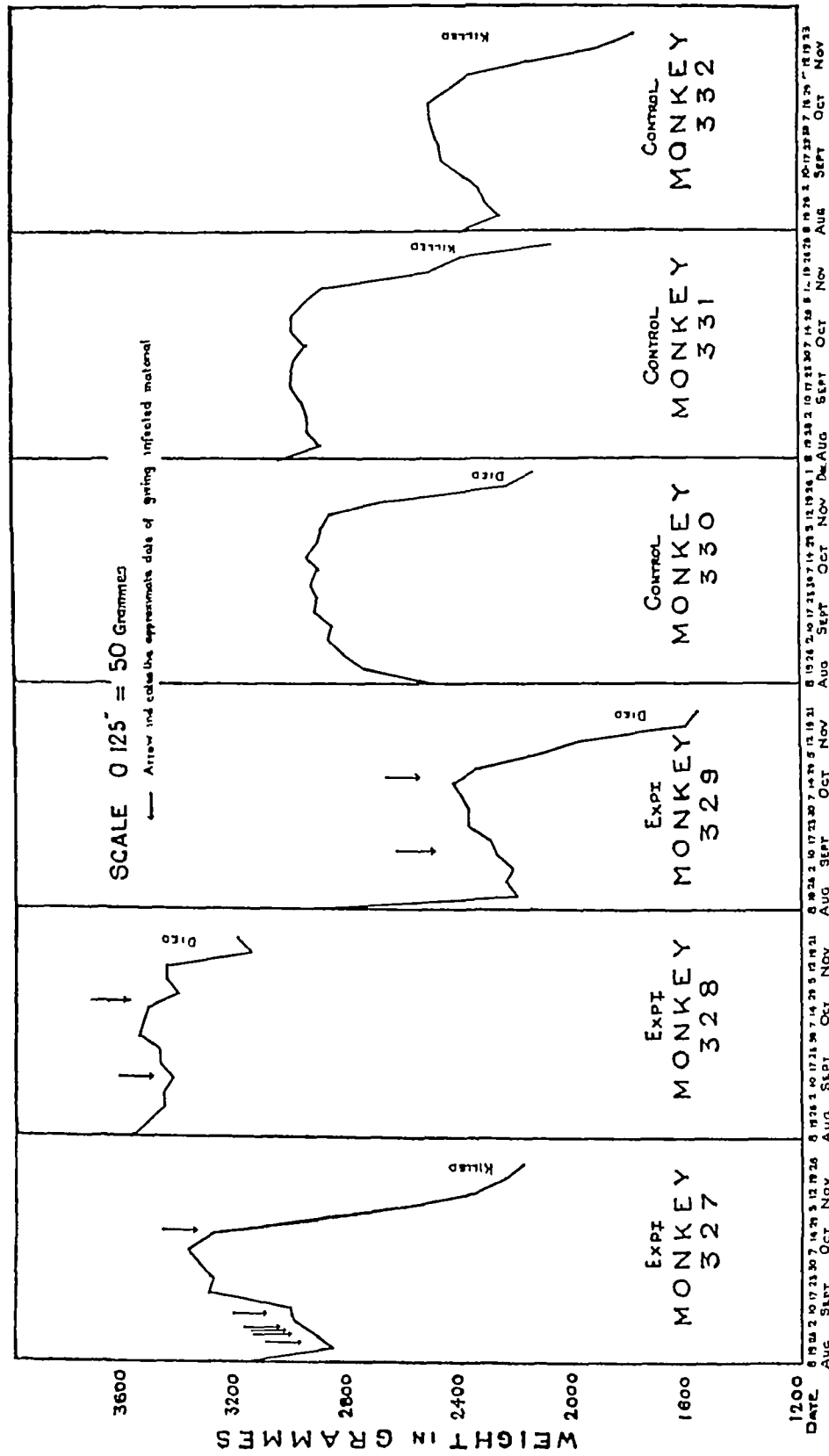
30th August 1927       "       "       "       (after passage through animals)

6th September 1927   "       "       "

1st September 1927   "       "       "

17th October 1927, saline suspension of sprue fæces

CHART II  
*Showing weights of experimental monkeys*



*Post-mortem*—The stomach, duodenum and upper part of small intestine were normal, but the lower part of the ileum was thickened and injected and the mucous membrane was soft and velvety and showed small hæmorrhages but no breach of surface to the naked eye. The cæcum and the rest of the large intestine were in a state of subacute dysentery. The mucous membrane was swollen and inflamed and standing out on its surface were marked conical and nipple-shaped elevations almost suggesting new growth. These elevations were capped with an ulcer or græenish slough. The rectum showed more general thickening of mucous and submucous coats without visible ulceration. This condition of the large intestine is represented in Plate I.

*Micro-histology*—The intestinal changes in this animal were more widespread as the small intestine shows degeneration and desquamation of superficial layers of mucosa with lymphoid hyperplasia and inflammatory reaction of the submucosa. The muscular layers stained diffusely with eosin and the nuclei were blurred and indistinct. The changes in the large bowel were still more extensive, the mucosa having sloughed in several places and the submucosa was infiltrated with macrophages and inflammatory cells. The muscle layers showed toxic degeneration, and masses of bacteria, mostly cocci, were lying in the mucosa and submucosa. The liver and kidneys showed advanced toxic poisoning and the spleen cells to a less extent. There was no excess of hæmosiderin in the liver.

### Monkey 329

Deficiency diet begun on 8th August 1927

Infected feeds—

12th September 1927, saline suspension of sprue fæces (Gomez)

17th September 1927     "           "           "           "

*Clinical Course*—Original weight of the animal was 2,910 grammes and fluctuated till the middle of October, after which it fell almost vertically till its death on 21st November 1927, when its weight was 1,560 grammes. It commenced to show signs of scurvy on 30th September 1927, swelling and ecchymosis of eyelids, and scorbutic gums. No signs of diarrhœa or dysentery were present up to the time of its death.

*Bacteriological Examination*—The animal died during the night and showed signs of decomposition. No cultures were made.

*Post-mortem*—The upper part of the gut was apparently normal, but the whole of the large intestine was in a state of subacute dysentery with much thickening of mucous and submucous coats and a few small ulcers and punctate hæmorrhages and thick tenacious mucus. Decomposition was evident so the organs were not kept for section.

be rarely as high as 60. This factor incidentally may have some bearing on the infrequency of organisms of the dysentery group which are known to die out quickly in acid stools. Faecal spirochaetes were rarely present. Yeasts were almost invariably present together with fat globules. Charcot-Leyden crystals were not seen. The faeces were then plated out on agar, Conradi and McConkey plates and fished after 48 hours in the usual way.

The results are grouped in Table I into three series of sprue cases, Nos I and II were done in 1924 and 1925 (when the investigation ceased owing to the absence of the senior author), and the third series (since his return) have been conducted on more thorough lines.

TABLE I

TYPE OF STOOL	Number of stools	LACTOSE NON-FERMENTERS			
		Eberthella		Salmonella	
		No	Per cent	No	Per cent
Normal stools	10				
'Diarrhoea'	36	1	3	13	36
'Dysentery'	89	46	52	20	22
Sprue Series I and II	47	12	26	11	23
Sprue Series III	51	16	31	21	41

Further details of Sprue Series I and II have already been summarised by us in a 'Progress Report on Sprue' (Transac 6th Congress, F E A T M, 1925). The results were similar to those obtained in the present series and will not be referred to again here.

The third series of sprue cases collected during the last 14 months (December 1926—February 1928) will now be considered in detail.

#### FÆCES

51 samples (from about 36 patients)

#### ÆROBIC ORGANISMS

*Cocci* either Gram negative or positive, chiefly the latter, were found in most cases, and showed the usual biological reactions. Most of them would be described as enterococci.

#### BACILLI

*Lactose fermenters*—These were of course present in all cases and selected colonies from each case were studied for unusual reactions but most strains were found to fall into the *B. coli* group.

*Lactose non-fermenters*—*B. faecalis alcaligenes* was recovered eight times out of 30 examinations. The lactose non-fermenters were divided by their biochemical reactions into

I *Eberthella*, and

II *Salmonella*,

and their characters and affinities are given in Table II.

The cæcum was ulcerated and showed the changes found in dysentery. One ulcer in the head of the cæcum had perforated and an abscess had formed and had become adherent to the liver which was infiltrated at the area of contact. The lymph glands were enlarged and matted together around the abscess. Other small ulcers were present in the lower part of the large bowel.

*Micro-histology*—The changes here were limited to the large intestine, particularly the cæcum, the mucous membrane of which was in a state of homogeneous degeneration as if infiltrated with amyloid substance (which did not give the amyloid reaction). The normal structure and staining reaction of this part of the gut was lost. A condition of toxic necrosis was present at other levels in the large intestine and the parenchyma of the liver, kidney and mesenteric glands was similarly affected. There was increase of iron in the liver. There was an abscess extending into the liver substance at one place, but bacteria were not detected in this area.

#### *Control Monkey 332*

Deficiency diet begun on 8th August 1927

No infected feeds

*Clinical Course*—Maintained its weight at about 2,400 grammes till end of October, when it began to fall rapidly, reaching 1,790 grammes at death on 23rd November 1927. On 15th October 1927, began to show signs of incipient scurvy, ecchymosis and swelling of the eyelids. The gums were noted to be scorbutic on 5th November 1927. The animal was put on orange juice in the later stages but it did not improve. No diarrhoea or dysentery were noted during life.

*Bacteriological Examination*—Not done

*Post-mortem*—Portions of the large intestine showed slight inflammatory signs with submucous hæmorrhages and thickening of mucosa. No ulcers were seen. The remaining organs were normal, but hæmorrhages were found in the muscles and other signs of scurvy.

*Micro-histology*—The changes in the large bowel were of a slight nature and showed early degree of toxic degeneration without signs of inflammation. The principal changes were in the superficial mucosa.



It is remarkable that in this series of 51 samples not once was a recognised pathogen isolated. One strain gave the sugar reaction of Flexner's dysentery bacillus but was motile and strongly hæmolytic, and did not show any agglutination with Flexner antiserum, another resembled Strong's dysentery bacillus but was motile and produced acid and clot in milk. The other suspects were Morgan's bacillus and organisms like *Asiaticus*, *Carolus*, *Columbiensis* and others of doubtful pathogenicity.

#### DUODENAL FLUID

Obtained during life by the passage of a duodenal tube from eight sprue cases

#### Summary of results

(a) Streptothrix	2 strains
(b) Cocci	16 "
(c) Gram negative bacilli—	
(1) Coliform	14 "
(2) Non-coliform	2 "
(d) Gram positive bacilli—	
(1) Spore bearers (aërobic)	4 "
(2) Spore bearers (anærobic)	Nil
(3) Non-spore bearers	14 strains
Cocci Sixteen strains isolated	
<i>Staphylococci</i>	9 "
(All Gram positive)	4 hæmolytic
	5 non-hæmolytic
<i>Diplococci</i>	7 strains
Gram positive	5 "
Gram negative	2 "
Hæmolytic	3 "
Non-hæmolytic	4 "

Types of bacilli found in the duodenal contents of the different patients —

Case 1 —Streptothrix—cocci—full lactose fermenters (AG)—Partial lactose fermenters (A—)—Lactose non-fermenters (—)

Case 2 —Cocci and AG, A—, —, groups

Case 3 —Cocci and A—, —, groups

Case 4 —Streptothrix cocci and A— group

Case 5 —Cocci and A— group

Case 6 —A— and — groups

Case 7 —Cocci, A— and — groups  
(Gastric contents)

To put the case more briefly —

The duodenal contents were never sterile

One case yielded five types of organisms

One case yielded four types of organisms



TABLE III—(contd)  
Bacilli Gram negative coliform

Strains	Motility	Lactose	Glucose	Mannite	Dulcitol	Sucrose	Milk	Indol	Voges-Proskauer	Hemolysins	Affinities
<i>Full lactose fermenters</i>											
1	—	AG	AG	AG	AG	AG	AC	—	+	—	} <i>B coli communis</i>
2	+	AC	AG	AG	—	AG	AC	+	—	—	
3	+	AG	AG	AG	—	A—	AC	+	—	—	
<i>Partial lactose fermenters</i>											
4	+	A	AG	AG	—	—	A	+	—	—	} <i>B meta dysentericus</i>
5	—	A	A	—	—	A	—	—	—	—	
6	+	A	A	—	—	A	AC	—	—	—	
7	+	A	A	A	—	A	—	—	—	—	
8	—	A	A	A	—	A	—	—	—	—	
9	+	A	A	A	—	A	—	—	—	—	
10	—	A	A	A	—	A	—	—	—	—	
11	+	A	A	A	—	A	—	—	—	—	
12	—	A	A	—	—	A	—	—	—	—	
13	+	A	A	—	—	A	—	—	—	+	
<i>Lactose non fermenters</i>											
14	—	—	—	—	—	+	—	—	—	—	} <i>B faecalis alcaligenes</i>
15	+	—	—	—	—	—	—	—	—	—	

Bacilli Gram positive aerobic non-spoilage bearing

Strains	Motility	Lactose	Glucose	Mannite	Dulcitol	Sucrose	Milk	Indol	Voges-Proskauer	HÆMOLYSINS	
										On blood agar	Suspensions of R B C
Hemolytic											
16	+	A	A	A	-	A	CD	-	+	+++	+++
17	-	-	-	-	-	-	-	-	-	++	+
18	-	A	A	A	-	A	AC	-	-	++	+

#### EXPLANATION OF PLATE XI

- Fig 1 Low power magnification of ileum of monkey showing coagulation necrosis of superficial mucosa, and of large areas in the submucous layer
- „ 2 Cæcum of Monkey 331 showing partial destruction of mucous layer and fibrotic changes in submucous layer
- „ 3 Cæcum of Monkey 281 showing earlier degenerative changes in mucous membrane
- „ 4 Cæcum of Monkey 331 showing changes similar to those in Fig 2
- „ 5 Large intestine of Monkey 327 showing coagulative necrosis of mucous membrane
- „ 6 Duodenum of Monkey 332 showing similar changes with early sloughing of mucous membrane and irritation and thickening of submucous layer

not clot milk. Five of these appear to be identical and are probably *B. meta-dysentericus* (Cast) Species No 7 was present in three samples. It produced acid only in lactose, glucose and sucrose, no change in milk, a non-indol producer, and was non-hæmolytic.

(c) *Gram negative coliform lactose non-fermenters*—Only two such were isolated and were probably *B. faecalis alcaligenes* (non-hæmolytic).

(d) *Gram positive, non-sporing, non-coliform organism*.—This is a mixed group very difficult to analyse. Three were hæmolytic. Two of these gave identical reactions, viz., acid in lactose, glucose, mannite and sucrose, clot in milk, neither indol or Voges-Proskauer reaction and both were small thin bacilli. The third hæmolytic strain was negative to all the biochemical reactions. Three other strains gave biochemical and morphological reactions as above but were not hæmolytic. The identity of this group of six strains has not been determined but they resemble *B. acidophilus*.

Another group of strains gave the following reaction—Acid in glucose with or without acid in dulcitate and sucrose, digestion in milk, no indol or Voges-Proskauer. These were non-hæmolytic. This group also resembles *B. acidophilus*.

*Gram positive aerobic spore bearers*.—Two of the four strains isolated were hæmolytic and their other characters are seen in Table III.

*Streptothrices*.—Two strains were isolated and were both strongly hæmolytic.

*Anærobic bacteria*.—None were isolated from any of the duodenal contents.

*Remarks on duodenal contents*.—The point of interest is the number and variety of strains isolated from these specimens of duodenal contents. Out of the fifty-two strains fifteen were hæmolytic which were distributed in 5 out of the seven cases. The acidity or otherwise of the stomach contents is unfortunately not known for these particular cases\* but it has been shown that absence of HCl in the gastric juice in pernicious anæmia has a marked influence on the bacterial content of the duodenum which under such circumstances is presumably infected from upper levels of the alimentary tract. The absence of *B. coli hæmolyticus* and of anærobes like *B. welchii* is important. The presence of an unknown organism of peculiar characters in the duodenal contents and in the circulating blood of two other cases will receive separate treatment later.

#### HÆMOLYTIC BACTERIA.

Hæmolysins were tested for in three ways—

(1) For the presence of free hæmolysin in saline suspensions of the fæces

\* In a previous analysis of the stomach contents of 26 cases of sprue we found (Fairley and Mackie) —

Achlorhydria in	7 cases
Hypochlorhydria in	8 "
Normal acidity in	7 "
Hyperchlorhydria in	4 "

indicating a deficient acidity in more than half the cases

PLATE XII

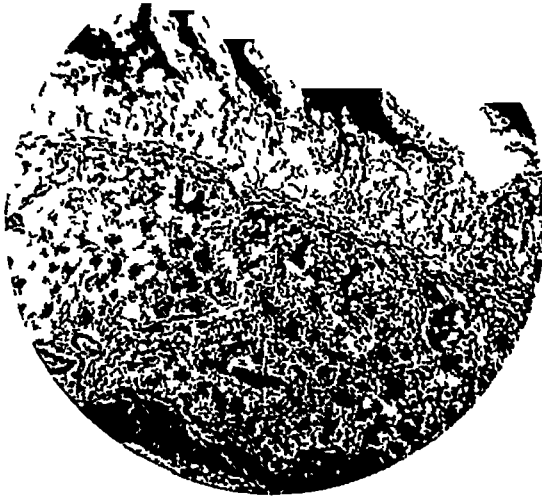


Fig 1



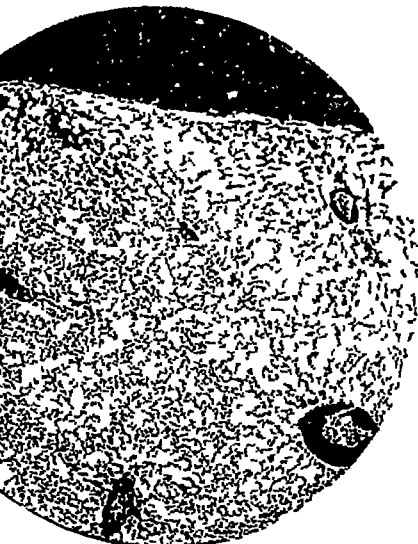
Fig 2



Fig 3



Fig 4



separate cases were strongly hæmolytic. It is very difficult to assess the importance of this hæmolytic function of bacteria in sprue as the results are only qualitative and sufficient information as to the presence of such bacteria in normal fæces in the tropics is wanting.

## DISCUSSION OF THE LITERATURE

We have not been able to find any papers dealing in detail with the identification of intestinal bacteria in sprue though several references have been made to the presence of bacteria of the dysentery group. Some writers, on very slender grounds, have assumed that sprue is a chronic form of dysentery or at least that such organisms are frequently to be found in the fæces of sprue patients. We are not aware of any such careful study of the problem as would justify this belief and our own investigations which have been carried on over a period of four years lends no support to this hypothesis.

It is outside the purpose of this paper to traverse the enormous literature which exists on bacterial flora of the intestinal tract, or to attempt to produce order out of the chaos which surrounds our present state of knowledge, but we have paid particular attention to some of the more recent researches on the intestinal flora in pernicious anæmia. This we do, not because we believe that the diseases are identical as some argue, but because the problem of the two diseases intersects at least at one point, namely, the extensive blood destruction which takes place, and which in both may well originate in the intestine.

The bacteriology of the duodenum as a specialised part of the intestinal tract which is accessible by modern methods of investigation is of much interest and we must first enquire what is the normal bacteriology of this part of the intestinal tube.

Kendal, Day, Walker and Haner (1927), as a result of the study of 50 normal cases, came to the following conclusion —

'Half the number failed to yield a significant number of organisms in culture, that is, they were nearly or completely sterile. The remainder varied from very moderate cultivations yielding bacteria that were chemically inert except for acid production to heavy growths reminiscent of those that would have confidently been anticipated had the specimens come from lower levels.' The direct microbicidal action of the duodenum which has been claimed by many observers is not supported by these results. They found that the relative abundance of the mucosus capsulatus group and of enterococci and staphylococci suggest that such bacteria are easily adaptable to this environment. Three cases out of the 50 showed the presence of gas bacilli (*B. welchii*) which disposes of the view that this organism is entirely relegated to the lower levels of the intestinal canal. These authors quote the observations of numerous earlier workers on this subject who came to the conclusion that the duodenum was sterile in health, and that, of late, observers, who, using the duodenal tube, found that a number of different organisms particularly cocci, gram positive and negative lactic acid producers and the mucosus capsulatus group, were present in normal duodenal contents.





contents of the duodenum have a tendency to become faecal in type The table summarising his finding is reproduced

Occurrence of Bacterial Types in the Duodenum

Gastric Resting Juice	No of cases	Coliform Bae		Staphylococci		Streptococci		Gr + Anaerobes		Gr + Aerobes		Sterile	
		No	Per cent	No	Per cent	No	Per cent	No	Per cent	No	Per cent	No	Per cent
Normal or Hyperchlorhydria	125	9	7	43	34	8	6	10	8	19	15	08	86
Hypochlorhydria	32	16	50	21	64	4	13	3	10	5	16	3	10
Complete Achlorhydria	19	14	90	36	73	34	69	15	30	16	32	2	4

Referring to the question of hæmolytic strains, he finds a great increase of the hæmolytic form of *B coli* (48 per cent as compared with 8 per cent in normals), of streptococci (4 per cent compared to 2 per cent), staphylococci (20 per cent compared to 16 per cent) and anaerobes (24 per cent compared with 18 per cent in normals) That is, all hæmolytic strains were much increased but particularly those of *B coli* He makes a remark in conclusion which, though applied to pernicious anæmia, may be equally applicable to the anæmias of sprue He thinks the failure to incriminate any particular bacterium as the cause of anæmia may be because there is no such specific agent, the toxins being the product of grossly faecal flora which comes to occupy the small intestine and in which one may find varving mixtures of hæmolytic and neurotoxic strains

An important paper by Moench, Kahn and Torrey (1925) on the faecal flora of pernicious anæmias conveys much information and their summary is given *in toto* —

‘A bacteriologic analysis of 72 stool specimens from 33 cases of pernicious anæmia of various durations showed in practically every case an unusually large number of viable organisms, of which *B coli*, streptococci, *B welchii* and, at times, *B acidophilus* were the most prominent types These findings indicated that the flora of the large intestine is of an actively growing, non-proteolytic fermentative type’

‘The most significant feature revealed by these examinations would seem to be the uniformly high counts for *B coli* and *B welchii* The numbers of both these organisms averaged much higher than for normal persons or for other pathologic conditions Although streptococci were also very numerous, they conformed to the normal intestinal types, no representative of the hæmolytic group being encountered’

‘Pure cultures of *B welchii* strains were isolated from 26 of these cases, and subjected to differential tests Representatives of the 4 fermentative types of Simond were encountered, but type 1 occurred with the greatest frequency

#### EXPLANATION OF PLATE XIII

- Fig 1 Shows extensive toxic necrosis of the renal tubules
- „ 2 Shows changes in Lieberkuhn's follicles of large intestine at the edge of a necrosed area
- „ 3 Shows the invasion of the mucosa at the margin of a necrotic area by inflammatory cells The glandular elements are almost completely destroyed
- „ 4 Shows the invasion of the deeper layer of the submucosa in the affected part of the intestine by numerous bacteria
- „ 5 The invasion of a villus by slender curved bacilli (magnification about 1,000)
- „ 6 Similar bacilli (magnification about 3,000) lying in the submucous layer of large intestine These bacilli during life showed a slow serpiginous motility and could not be recovered by culture

5 Hæmolytic gas producing anærobes were not examined for in all cases but were invariably present when suitable cultures were made. As these organisms are almost invariably present in normal stools the mere fact of their presence in sprue stools cannot in itself be claimed as significant.

6 The duodenal contents of eight cases received particular attention. None were sterile. One case yielded five types of bacteria, one case four, three cases three, and two cases yielded two types of bacteria. A hæmolytic streptothrix was present in two cases. Hæmolytic strains of *B. coli* were not isolated, nor were anærobes of *B. welchii* types found in any of the eight samples.

7 Half the strains of cocci were hæmolytic and a number of strains of hæmolytic Gram positive aerobic bacilli were isolated from the duodenal contents.

8 We may conclude that the duodenal contents of sprue cases are rich in bacteria and that a number of these are powerful hæmolytics. None of these could be definitely identified with the recognised pathogenic bacteria which are found in the intestinal canal in other diseases.

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PLATE XIV

CÆCUM

OF

EXPRIMENTAL MONKEY No 328



the blood actually occur, an increase renders the blood-stream more favourable, and a decrease less favourable, as a habitat for the malarial organisms, so that relapses in malaria may be due to such changes

Rudolf and Marsh (1927) found that during malarial therapy the administration of glucose had no effect on the number of parasites, while insulin produced indefinite effects

Savino (1927) says that Fleig observed that glucose stimulated the division of *T. brucei* and of *T. lewisi*, and that the injection of insulin brings about a diminution of glucose with, at the same time, a decrease in the number of trypanosomes. Following an injection of glucose alone there was an increase of 120 per cent in the number of parasites

The results of the work quoted above favour the view that a condition of hyperglycæmia was favourable to the multiplication of parasites in the body

(b) *Blood sugar during fever*—Boulay and Leger (1923) state that during a malarial attack there is an increase of the blood sugar, but without glycosuria. This slight hyperglycæmia lasts only two to five days

Monteleone (1925) found that in acute malaria the cerebrospinal fluid contained more sugar than normal, but was unaltered in chronic malaria

Peterson (1926) determined that in the fasting patient the period of the chill and rise in temperature of malaria fever is associated with a decrease in the sugar level. This corresponds to the time of a peripheral leukopenia and a diminished concentration of blood protein. With the crisis and fall in temperature, blood sugar increases, leucocytes increase in the peripheral blood-stream and the blood proteins increase

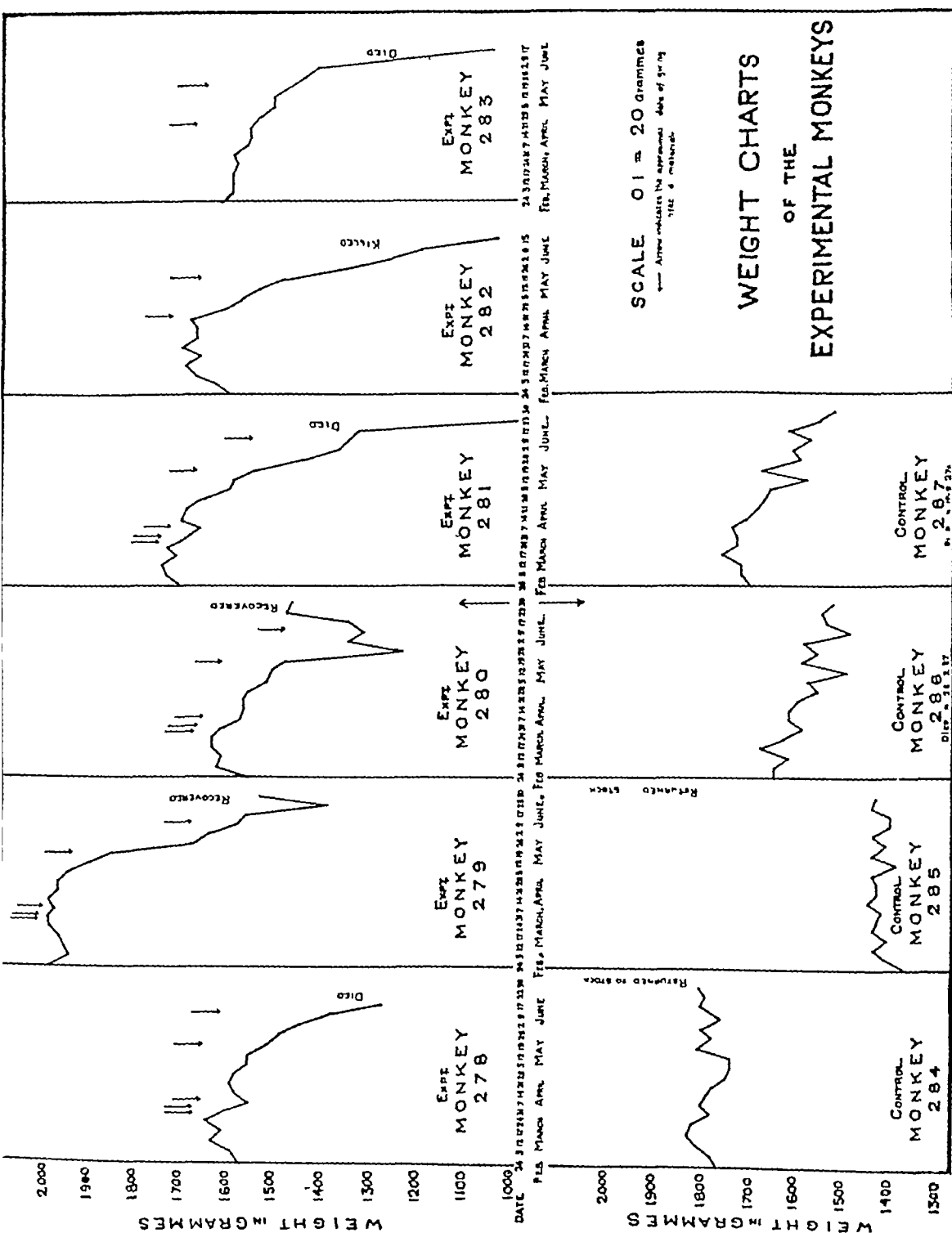
Rudolf (1926) states that Marsh found that the blood sugar decreased during the malarial fever, returning to normal afterwards, and suggests it might be that the glucose content of the blood had relation to comparative immunity to malaria, and to the observation that the undernourished did not invariably develop clinical malaria

Rudolf and Marsh (1927) found that during malarial therapy, the blood sugar was found to vary inversely with the temperature. The administration of glucose during malarial pyrexia did not produce an obvious change as regards the objective signs, but apparently brought relief of the subjective symptoms. The administration of insulin during malarial therapy terminated the fever in 60 per cent of instances. Relapses followed but differed from those following small doses of quinine in being of a low degree of pyrexia. The lowering of the blood sugar would not appear to be the cause of the cessation of the fever

Brems (1926) found that adrenalin given by the mouth in sufficient doses (4 milligrams) produces a marked and constant hyperglycæmia. Sinton (1926) used adrenalin as a provocative agent in the diagnosis of uncured malarial infections, and found that 16.6 per cent of uncured infections of malaria relapsed inside two hours after the injection and 32.4 per cent in one week

These observations show that in malaria there is a change in the sugar content of the blood. Some workers think that a decrease in blood sugar has a relation to the absence of relapse in the disease, and that an increase brings relief to the subjective symptoms of the paroxysm

CHART I  
Showing weights of experimental monkeys.



would even be beneficial, while after the acute symptoms had disappeared it might tend to cause relapse

### EXPERIMENTS AND RESULTS

The patients treated were young British soldiers suffering from chronic benign tertian malaria, as diagnosed by the thick film method of microscopical examination.

During the trials of a special line of quinine treatment for benign tertian malaria in the malaria treatment centre at Kasauli alternate cases were given three ounces of sugar mixture containing two ounces (56.6 grammes) of sugar once daily, throughout treatment and during eight weeks observation after the end of all treatment. The quinine treatment consisted of a mixture containing quinine sulphate, citric acid and magnesium sulphate given twice daily in doses containing 15 grains (1 gramme) of quinine to each dose for the first fourteen days of treatment, and then one dose containing 10 grains (0.6 gramme) of quinine given once daily for six weeks. The amount of alkaloid in the solution was always tested before administration to see that it contained the amount prescribed (Sinton, 1925).

After the commencement of treatment the blood of each patient was examined once weekly during treatment, and weekly during an observation period of eight weeks after the end of all treatment. The first weekly examination during observation was always done on the day following the last day of treatment (Sinton, 1926).

'Relapses' were diagnosed by the finding of malaria parasites in the peripheral blood by the thick film method of examination, during an observation period of eight weeks after the end of all treatment. Patients who did not 'relapse' during this period were considered as 'cured' (Sinton, 1926).

*Treatment—Q C M*—Forty-one patients were given the above treatment without the administration of sugar mixture. Six of these had splenic enlargement before the commencement of treatment. Of these twenty (48.8 per cent) relapsed within an observation period of eight weeks after the end of all treatment. All these relapses were due to *P. vivax*. These relapses were detected at the following periods of observation—One in each of the seventh, eighth and ninth weeks, two in the fourth week, three in the sixth week, and six in each of the second and third weeks.

*Treatment—Q C M S*—Twenty-eight patients were given the above quinine treatment and in addition received a daily dose of three ounces of a sugar mixture containing two ounces of sugar. Of these two had splenic enlargement before the commencement of treatment and one at the end of treatment. One patient was lost sight of before the completion of observation and 19 had relapses due to *P. vivax*. The relapse rate was, therefore, a possible maximum of 71.4 per cent, with an observed minimum of 67.8 per cent. These relapses were detected at the following periods of observation—One in the fourth week, two in the eighth week, five in the third week, and eleven in the second week.

# THE BACTERIOLOGY OF SPRUE

BY

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AND

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(Being Part VIII of the Sprue Researches at the Haffkine Institute)

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CULTURES of the circulating blood taken during life from about thirty cases of sprue were made on agar, blood agar, broth and an acid glucose broth (for monilia). In a few cases anærobic cultures were also made. In two cases only were bacteria found and a similar organism was found in the duodenal contents of an early and acute case of sprue.

This bacterium which we have provisionally named 'B gomezi' (Mackie) will be the subject of a separate paper. With these exceptions the blood was always sterile and the use of enrichment method for monilia has not shown the presence of these organisms.

Fresh citrated blood from most of the above series was examined by the dark-ground method for spirochætes, etc., but no such organisms have ever been demonstrated.

Since the sprue investigation began in 1924 we have studied 92 specimens of stools and six of duodenal contents from about sixty cases of sprue at different stages of the disease. We hoped by studying the various intestinal organisms to arrive at a picture of the bacterial flora and to see if and how it differed from other cases of bowel disease and we hoped to find some one organism constantly recurring which might be definitely associated with the disease. If these hopes have not been realised we may nevertheless regard the results as instructive if only for their negative character, though the association of one peculiar organism to be described later may turn out to be of some significance.

The stools were brought direct from the hospital to the laboratory as fresh as possible and were examined in fresh and stained films. The scarcity of protozoa was a remarkable feature as on no single occasion were *E histolytica* or its cysts found and only rarely *E coli*. Similarly flagellates were only seen once or twice throughout the investigation and this is probably attributable to the high degree of acidity of sprue stools. We found the pH to be often as low as 3.5 and to



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TABLE II

GRAM NEGATIVE COLIFORM LACTOSE NON-FERMENTERS

GRAM NEGATIVE COLIFORM LACTOSE NON-FERMENTERS														Affinities
Genus	Motility	Glucose	Lactose	Mannite	Maltose	Sucrose	Dulcile	Litmus milk	Voges Proskauer	Koser's citrate	Indol	H <sub>2</sub> S	Hæmoly- sis on blood agar	
EBERTHELLA	1	A	—	—	—	—	—	—	—	+	—	—	—	<i>E chyligena</i> (Ford)
	2	A	—	—	A	—	—	—	—	—	+	—	—	?
	3	A	—	A	A	—	—	—	—	+	+	—	+	<i>B dysentericæ</i> (Flexner)
	4	A	—	A	A	A	—	AC	—	—	+	—	—	<i>B dysentericæ</i> (Strong)
	5	A	A	A	—	A	—	—	—	—	—	—	+	<i>B acidophilus</i> (except for Grams staining)
	6	A	A	A	A	A	—	—	—	—	—	—	+	<i>B madampensis</i> (Cast)
	7	A	A	A	A	A	—	A	—	—	+	—	+	<i>E pyogenes</i> (Passet)
	8	A	A	A	A	A	A	A	—	—	+	—	—	
	9	AG	—	—	AG	AG	—	AG	—	—	—	+	—	<i>B enteritidis</i>
SALMONELLA	10	AG	—	—	—	—	—	—	—	—	—	+	—	<i>B morgan</i>
	11	AG	—	—	—	—	—	—	—	—	+	+	+	<i>B morgan</i> (hæmolyticus)
	12	AG	—	—	AG	—	—	—	—	—	+	—	—	<i>B carolinus</i> (Cast)
	13	AG	—	—	AG	—	AG	—	—	—	+	—	—	<i>B columbiensis</i> (Cast)
	14	AG	—	—	AG	AG	—	AC	—	±	+	±	++	<i>B asiaticus</i> (hæmolyticus)
	15	AG	A	A	AG	—	—	—	—	—	+	—	—	?
ESCHERICHIA	16	AG	A	AG	AG	AG	AG	AC	—	—	+	—	—	<i>B pseudo asiaticus</i> (Cast)
	17	AG	A	A	AG	AG	—	AC	—	—	+	+	—	?

A Gram positive aerobic spore bearing bacillus strongly hæmolytic (? *B subtilis*) was also isolated from some stools

A Gram positive aerobic spore bearing bacillus strongly haemolytic (? *B. subtilis*) was also isolated from some stools

extract was found nearly all soluble in water. The ether extract consisted of chlorophyll and two resins, the resin insoluble in alcohol forming the large proportion. The resin soluble in alcohol was readily saponified and left a tingling sensation in the throat. The aqueous solution of the substance soluble in alcohol had an acid reaction, it gave no coloration with ferric chloride, showing the absence of tannin. It fused at about  $60^{\circ}\text{C}$  into a blackish mass. Heated in a test tube it gave off fumes of creosote. It was precipitated by lead acetate and on removing the lead by  $\text{H}_2\text{S}$ , a reddish substance was left behind, which the author called gymnemic acid. The yield of this acid having the formula  $\text{C}_{32}\text{H}_{55}\text{O}_{12}$  was about 6 per cent. This acid was described to be a glucoside as it was hydrolysed by dilute hydrochloric acid and the resulting liquid reduced Fehling's solution. It was stated to be responsible for the effect produced on the nerves of taste. When an alkaline solution of the leaf was mixed with chloroform, a crystalline residue of a brownish colour was left behind which had a bitter taste and acted as a sialogogue. Although it gave more of the nature of coloured precipitates with alkaloidal reagents, it was a neutral principle. Hooper also got pararabin and calcium oxalate from the acid extract of the leaves.

Power and Tutin (1904) next took up the subject and made a thorough investigation of the leaves. They purified the acid by extracting it with chloroform. The residue was dissolved in alcohol, dried and then extracted with ethyl acetate and, finally, decolorized by animal charcoal. The purified acid was soluble in caustic alkali and precipitated by mineral acids. When boiled with 20 per cent KOH or with HCl or  $\text{H}_2\text{SO}_4$  for a short time it lost its antisaccharine property. These workers did not agree with Hooper regarding the solubility of gymnemic acid in ether, benzene or chloroform and they stated that the acid was not a glucoside as it did not give any sugar on hydrolysis. By fusion with KOH, they got protocatechuic acid and p-oxy-benzoic acid. On oxidation with potassium permanganate formic acid was obtained.

In order to test the effect of the drug on blood sugar in rabbits, we prepared different fractions from the leaves and tested them separately. The petroleum ether extract amounted to about 4 per cent of the dry leaves, the ethereal extract was about 2 per cent and the alcoholic extract about 9 per cent. The water-soluble portion of the ethereal and of the alcoholic extracts were tested separately. Finally gymnemic acid, which is said to have the antisaccharic property, was isolated and purified by the following method —

Five hundred grams leaves were extracted repeatedly with rectified spirit 95 per cent, the solvent was recovered and the residue was then extracted with hot water when almost the whole of it went into solution. This solution was filtered and repeatedly extracted with ether. The ethereal solution on evaporation gave a black tarry product. The mother liquor, which had a bitter taste, was precipitated with dilute hydrochloric acid when crude gymnemic acid soon solidified weighing 13 grams. This was purified by extracting with ethyl-acetate and the extract was treated with small quantity of animal charcoal, filtered and finally the solvent evaporated. The sodium salt of the acid was then prepared by neutralising its solution in absolute alcohol with alcoholic NaOH. The dry salt was obtained as a light yellow powder.

Three cases yielded three types of organisms

Two cases yielded two types of organisms

Anærobic bacteria were never found

Table III shows the detailed characters of these

TABLE III  
*Strains of Bacteria Isolated from the Duodenal Contents*  
*Cocci Gram positive*

Strains	Lactose	Glucose	Mannite	Dulcitol	Sucrose	Milk	Indol	Voges-Proskauer	HÆMOLYSINS	
									On blood agar	Suspension of R B C
<i>Hæmolytic</i>										
1	A	A	A	—	A	—	—	—	++	++
2	A	A	—	—	A	AC	—	—	++	+
3	—	—	—	—	—	—	—	—	+	+
4	A	A	—	—	A	A	—	—	++	++
5	A	A	A	—	A	—	—	—	+++	+
6	—	A	—	—	A	—	—	—	+++	++
7	—	A	—	—	A	—	—	—	++	+
<i>Non hæmolytic</i>										
8	—	A	A	—	—	—	—	—	—	—
9	A	A	—	—	—	—	—	—	—	—
10	—	A	—	—	A	—	—	—	—	—
11	A	A	—	—	A	A	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—
13	A	A	A	—	A	A	—	—	—	—
<i>Gram negative non hæmolytic</i>										
14	—	A	—	—	A	—	—	—	—	—

simultaneously by being converted into sugar This may of course be possible in a well fed animal but to obviate this fallacy we carefully starved our experimental animals from 24 to 36 hours before the test

TABLE I

*Action of the aqueous extract of the powdered leaves of Gymnema sylvestre on rabbits*

(Strength 1 c c = 0.28 gramme of powdered leaves)

Date	Identification No. of rabbit	Dose injected	Blood sugar before	Blood sugar 2 hours after
			Per cent	Per cent
17-2-27	White—4	1 c c	0.097	0.084
	White—5	1 c c	0.080	0.074
22-2-27	White—6	1 c c	0.068	0.060
	White—7	1 c c	0.084	0.082
1-3-27	White—8	1 c c	0.080	0.106
15-3-27	White—9	2 c c	0.069	0.068
	White—10	2 c c	0.107	0.096
	White—11	2 c c	0.065	0.065

TABLE II

*Action of the alcoholic extract of Gymnema sylvestre on rabbits*

(Extracted with 95 per cent alcohol evaporated to dryness and then extracted with distilled water)

(Strength 1 c c = 1 gramme of powdered leaves)

Date	Identification No. of rabbit	Dose injected	Blood sugar before	Blood sugar 2 hours after	
			Per cent	Per cent	
30-3-27	Brown—1	1 c c	0.080	0.073	
	Brown—2	1 c c	0.061	0.060	
	Brown—3	1 c c	0.072	0.069	
	Brown—4		0.065	0.059	Control Distilled water injected
	Brown—6	2 c c	0.066	0.058	
	Brown—8	2 c c	0.085	0.087	Got an attack of severe convulsions and died within 3 minutes

TABLE III—(concl'd)

Bacilli Gram positive aerobic non-spore bearing

Strains	Motility	Lactose	Glucose	Mannite	Dulcitol	Sucrose	Milk	Indol	Voges-Proskauer	HÆMOLYSINS	
										On blood agar	Suspensions of R B C

## Non hæmolytic

20	—	A	A	A	—	A	D	—	—	—	—
21	+	A	A	A	—	A	AC	—	—	—	—
22	+	A	A	A	—	A	AC	—	—	—	—
23	—	—	A	—	—	—	Alk	—	—	—	—
24	—	—	A	—	—	—	D	—	—	—	—
25	—	—	A	—	A	—	D	—	—	—	—
26	+	—	A	—	—	A	D	—	—	—	—
27	+	—	—	—	—	—	—	—	—	—	—

## Gram positive, aerobic spore bearers

28	—	—	A	—	—	A	D	—	+	++	+
29	—	—	A	A	—	A	—	+	—	++	+
30	—	A	A	—	—	A	A	—	—	—	—
31	—	—	A	A	—	A	—	—	—	—	—

## Streptothrices

32	—	—	A	—	—	—	D	—	—	+++	+
33	—	—	A	—	—	—	—	—	+	++	+

*Cocci*—All the strains isolated were Gram positive except two. These two produced acid in glucose and sucrose but no change on other sugars named nor in milk and were negative to indol and the Voges-Proskauer reaction. They were also non-hæmolytic.

The Gram positive group were either staphylococci or diplococci and showed the biochemical reactions as in Table III. Six out of the 14 were hæmolytic.

*Bacteria* (a) *Gram negative coliform lactose fermenters* (AG)—These represented *B. coli* and its near congeners. Some were indol producers. All were non-hæmolytic.

(b) *Gram negative coliform producing acid but not gas in lactose* (A)—These organisms (with one exception) produced no gas in any sugar and did

ACTION OF *Gymnema sylvestre* IN DIABETES

We also tried the drug in a number of cases of diabetes mellitus in order to see if it produced any reduction in the amount of sugar present in the blood or urine. The records of our observation in 6 such cases are given below. All these were uncomplicated cases of diabetes and were kept in hospital under strict observation. They were all placed on a fixed diet which was strictly under control. The total quantity of urine passed in 24 hours was carefully collected, measured and a portion of it was examined every day for the quantity of sugar present. The sugar content of the blood was also estimated from time to time and the figures given in the tables represent the 'fasting' level of blood sugar. The patients were regularly weighed during the course of treatment.

Of the 6 cases treated, 4 were given finely powdered leaves of *Gymnema sylvestre* in doses of one drachm of the powder three times a day. The total intake per day was thus 12 grammes or 180 grains of the powdered leaves.

## CASE No 1

Name—H. R. S., I Ch, male, 32 years

Date	Volume of urine in ccs	Sugar per cent	Total sugar excretion in gms	Blood-sugar per cent	Sugar value in diet in gms	Body weight in lbs	REMARKS
13-9-26	720	3	21.6	0.172	203	87½	Powdered leaves of <i>Gymnema sylvestre</i> , one teaspoonful three times a day
14-9-26	900	2.4	21.6				
15-9-26	1,200	2.5	30				
16-9-26	1,140	3.3	37				
17-9-26	1,140	3.1	35.3				
18-9-26	1,200	2.5	30.0			88	
21-9-26	1,140	2.5	28.0				
22-9-26	1,140	2.4	27.3				
23-9-26	1,200	2.0	24.0				
24-9-26	1,440	1.8	25.9				
25-9-26	1,140	1.7	19.3	0.168			Pulv. G. S. discontinued
27-9-26	720	2.2	15.8			90	
28-9-26	960	2.0	19.2				
29-9-26	960	2.0	19.2				
30-9-26	1,080	1.4	15.1				
1-10-26	1,020	1.5	15.3				

- (2) By subculture of all strains on blood agar
- (3) By bacterial washings of pure strains acting on saline suspensions of human red blood cells as a quantitative estimation

(1) We sought to ascertain the quantitative estimation of free hæmolysins in the fæces of sprue cases as compared with normal and non-sprue fæces. Our first case of sprue showed that dilution up to 1 in a million produced hæmolysis in 24 hours, and a subsequent examination of samples taken from different levels of the intestinal tract after death (duodenal, jejunal, ileal and colonic) all showed a high degree of hæmolysis. The examination of further cases of sprue and other diseases showed the results to be capricious and we were not able to exclude the action of other non-bacterial hæmolytics present in the fæces.

The origin of the high percentage of free hæmolysins in some cases of sprue fæces was uncertain and this method was then given up.

Methods (2) and (3) which gave respectively qualitative and quantitative degrees of hæmolysin were adopted but the latter has not been done on sufficient numbers of bacteria to justify any conclusions being drawn. The references to hæmolysin in this paper are concerned mainly with the qualitative evidence as to their presence. Even so this evidence is not complete as all strains of the fæcal bacteria were not tested for hæmolysis though this was done for all strains isolated from the duodenal contents.

*Hæmolytic power of (a) fæcal bacteria*—A few strains of *B. coli* were tested but none were found to produce hæmolysis. Such strains have been found in some cases of pernicious anæmia and have been credited with producing blood destruction. Amongst the strains of *Eberthella* and *Salmonella* shown in Table II, several were powerful hæmolytics, notably the *fleiner*, the *morgan* and *asiaticus*-like organisms. Gram positive spore bearers like *B. subtilis* were also found to be strong hæmolytics.

The anærobic were studied in a series of selected cases and in every case *B. welchii* was isolated and all the strains were strongly hæmolytic. Much attention has been paid to this organism as the possible source of hæmolysis in pernicious and other anæmia and this problem is equally important in sprue. The difficulties surrounding this subject are considerable and we only record the observation that this and other powerful hæmolytic bacteria are present in most if not all cases of sprue.

*(b) Duodenal contents*—The presence of hæmolytics in this position has been carefully examined from this view-point. Half the strains of cocci (which were presumably *staphylococcus* and *streptococcus pyogenes*) were hæmolytic, some very strongly so. Washings of cultures sometimes produced active blood lysis in dilutions up to one in a thousand. The lactose fermenters (whether partial or complete) showed no hæmolytic power nor did the *coli* group isolated from the duodenum.

Some strains of the Gram positive non-spore-bearing aerobic group were actively hæmolytic as were some of the Gram positive aerobic spore bearers. It is interesting to note that both strains of streptothrix in the duodenal fluid of



## CASE No 4

Name—J Adikari

D Y No 6

Date	Volume of urine in c cs	Sugar per cent	Total sugar excretion in gms	Blood-sugar per cent	Sugar value in diet in gms	Body weight in lbs	REMARKS
19-1-27	1,920	3	57.6	0.37	252	85½	Powdered <i>Gymnema sylvestre</i> leaves, one teaspoonful three times a day
21-1-27	1,800	3.3	59.4				
22-1-27	1,320	3.3	43.5			84½	
24-1-27	900	3.9	35.1				
25-1-27	1,320	2.7	35			84½	
26-1-27	1,260	3.2	40.3	0.358			
27-1-27	1,140	3.5	39.9			83½	
28-1-27	1,320	4.1	54.1				
29-1-27	1,140	3.8	43.3			83½	
31-1-27	1,200	4	48				
1-2-27	1,920	2.5	48	0.360			
2-2-27	2,108	2.9	60.9				

## CASE No 5

Name—J A

D Y No 6

Date	Volume of urine in c cs	Sugar per cent	Total sugar excretion in gms	Blood-sugar per cent	Sugar value in diet in gms	Body weight in lbs	REMARKS
12-2-27	1,800	3.7	66.6	0.256	252	81½	Alcoholic extract of the powdered leaves given. Dose equivalent to one tea spoonful of powdered leaves three times a day
14-2-27	1,500	3.6	54.0				
15-2-27	1,380	3.8	52.4				
16-2-27	1,800	1	72				
17-2-27	1,500	3.5	52.5			82½	
18-2-27	1,950	3	58.5				
19-2-27	1,500	4	60				
21-2-27	2,100	2.9	60.9				
22-2-27	1,500	4	60	0.283			
24-2-27	2,100	3	63			83	

Kendal and his co-workers give a tabular list of their findings (in 24 out of their 50 cases the remainder being sterile) as follows —

Organism	Times isolated	Percentage of cases
Mucosus capsulatus group (starch fermenting only)	14	28
Micrococcus ovalis	15	30
Staphylococcus	20	40
Streptococcus	8	16
Bacillus coli group (9, or 18 per cent were <i>B. acidilactici</i> )	14	28
Mesentericus group (aerobic, spore-forming, gelatin-liquifying)	12	24
Bacillus pyogenes foetidus	10	20
Yeast	7	14
Bacillus pyocyaneus	1	2
Bacillus welchii	3	6
Bacillus acidophilus	7	14
Alcaligenes group	6	12
Sarcinae	2	4

Bogendorfer (1922) found that at a distance of 1—2.5 meters from the pylorus two kinds of diplococci were found in healthy persons and that the colon bacillus was never found above a point 2.5 m. below the pylorus. In conditions of disease especially with an acidity of the stomach, organisms which are usually found only in the colon were discovered in the small intestine. They may be present, he adds, even in cases of hyperacidity.

Turning to the question of the intestinal flora (particularly the duodenal) in pernicious anaemia a great deal has been written and the following selections from the literature give the general trend of recent opinion.

Davidson (1927), writing on the bacterial content of the stools of pernicious anaemia with special reference to *B. welchii*, argues the importance of this powerful hæmolytic agent in the production of pernicious anaemia and concludes as follows —

(1) Clinical symptoms and pathological findings support the view that pernicious anaemia is caused by an absorption of toxin from the intestinal tract.

(2) A bacteriological examination of both small and large intestines shows a non-proteolytic fermentative flora which is typical for pernicious anaemia. A qualitative examination shows the organisms to be similar to those found in health. A quantitative examination shows an enormous increase of viable organisms particularly in regard to *B. welchii*.

(3) Biological and experimental evidence points to *B. welchii* as the most probable causal agent.

Knott (1927) in a paper on 'Addison's Anaemia: the rôle of achlorhydria and intestinal infection' finds that in cases of complete achlorhydria the bacterial

(2) The extracts made from the leaves of *Gymnema sylvestre* as well as gymnemic acid and its sodium salt have no effect on the blood sugar when given by subcutaneous injections to rabbits

(3) Powdered leaves and alcoholic extracts prepared from the leaves of *Gymnema sylvestre* have no effect on the blood or urine sugar of patients suffering from diabetes

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(50 per cent) These several strains differed also in the amount of hæmolysin produced Although most of them were strongly hæmolytic, it could not be said that they exhibited in general greater potency in this respect than did strains from normal human intestines All of the strains tested showed a high degree of pathogenicity, but this is also frequently true for strains from normal sources It would seem, then, that if these intestinal strains of *B welchii* are to be brought into etiologic relationship to pernicious anæmia, it must be on the basis of their excessive numbers and activities, particularly at levels of the intestine where absorption is active and where they are not commonly found or only in negligible numbers In view of Seyderhelm's finding that the flora of the large intestine tends to invade the small intestine in this disease, there is some ground for the latter supposition'

'Speculation is offered as to the possible significance of active growth of *B welchii* at the higher levels of the intestine in the production of the pernicious anæmia syndrome in view of its well-known capacity to elaborate a potent hæmolysin, an irritating acid and neurotoxic substances'

Dudgeon (1926) has collected a large amount of information on the intestinal flora under normal and abnormal conditions and his paper should be read in the original As it has no direct bearing on sprue or on the intestinal flora of the tropics it will not be summarised here He like many other recent writers lays stress on the fact that the intestinal flora varies considerably according to the dietary and this important factor needs to be emphasised in conditions such as sprue where the dietary plays so prominent a part in the treatment It is interesting to note that he only recovered *B welchii* in 35 per cent of his total cases, a figure which is much lower than we should have anticipated

A brief reference to the duodenal bacteriology of sprue is made in a paper by Van Steenis (1926) who examined three cases and found *B coli* in two cases and a streptococcus in the third

## CONCLUSIONS

- 1 The blood culture during life of thirty cases of sprue yielded an unknown bacillus in two The rest were sterile both to bacteria and to yeasts
- 2 Protozoa such as amœbæ and flagellates are excessively rare in sprue fæces—probably as a result of their high acidity
- 3 Ninety-two specimens of sprue fæces have been closely studied and a complete analysis of the last series of 51 cases is given in this paper
- 4 Not once in this series was a recognised pathogen isolated though an atypical strain resembling *B dysenteriae* (Flexner) was found once, *B dysenteriae* (Strong) once, and organisms like *B morganii*, *carolinus*, *columbiensis*, *asiaticus*, which are of doubtful pathogenicity, were each isolated on a few occasions \*

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\* The identification of the organisms referred to in this paper has been made according to Bergey's 'Determinative Bacteriology,' 2nd Edition, 1926, which has been taken as the standard work on this subject

cinchonidine were lævo-rotatory, he therefore suggested that the nomenclature was defective. After an experimental study on the toxicity of these alkaloids he came to the conclusion that cinchonidine was less toxic than cinchonine. He quotes Cushney as saying that cinchonidine is more liable to produce symptoms of poisoning, and remarks that Cushney evidently means cinchonine when he writes cinchonidine. Clerc and Pezzi (1923) observe that all the cinchona alkaloids paralyse the bulbar centre of the vagus. They further state that the acceleration of the heart caused by small doses of these drugs is due to excitation of accelerators and not to paralysis of the pneumo-gastric, which requires larger doses. Sollmann (1926), in his textbook on pharmacology, describes the action of cinchonidine and cinchonine as resembling that of quinine but says that the two former are endowed with less of the desirable effects and more of the undesirable side-effects. He further observes that cinchonine is 'much more depressant to the heart and more toxic than cinchonidine.

It will be seen, therefore, that a good deal of discrepancy exists between the findings of various observers. Some have credited less toxicity to cinchonine, while others signal it out as the most toxic. In this paper we propose to investigate the action of these two alkaloids on the heart.

#### THE CINCHONINE SERIES

Cinchonine  $C_{19}H_{22}ON$  occurs constantly in cinchona and cuprea barks, but the amount present is small and shows great variations. One of the best sources is *Cinchona micrantha* bark.

*Preparation*—After quinine sulphate has been separated, the mother liquor has caustic soda solution added to it, when the remaining alkaloids come down as a precipitate. The precipitate is boiled with successive small quantities of alcohol, and on cooling cinchonine crystallises out. The crude alkaloid is exactly neutralised with dilute sulphuric acid, and the sulphate recrystallised from boiling water.

*Properties*—Cinchonine behaves as a diacidic base and gives two series of salts. The neutral sulphate  $B_2H_2SO_4 \cdot 2H_2O$  forms rhombic crystals soluble in water 1 in 65.5 at  $13^\circ C$ . The acid sulphate  $BH_2SO_4 \cdot 4H_2O$ , colourless octahedral crystals, readily soluble in water 1 in 46 at  $4^\circ C$ . The neutral hydrochloride  $BHCl \cdot 2H_2O$  forms monoclinic crystals soluble in 22 parts of cold water. The melting point of the base is  $264^\circ C$ , and the optical rotation  $[\alpha]_D^{15} = +224.4^\circ$  in alcohol. The salts do not exhibit fluorescence in dilute sulphuric acid.

*Tests*—Cinchonine differs from quinine and quinidine in that it does not give the thalleoquin reaction and is not fluorescent in dilute sulphuric acid, and from cinchonidine in that it is sparingly soluble in ammonia solution and ether, and is dextro-rotatory.

#### THE CINCHONIDINE SERIES

Cinchonidine  $C_{19}H_{22}ON$  is an isomeride of cinchonine, and occurs especially in the bark of *C. succirubra*, *officinalis*, *tucujensis* and *lanceifolia*. The sulphate has largely been used to adulterate quinine as it is much cheaper.

# THE EFFECTS OF A DIET RICH IN SUGAR ON THE RELAPSE RATE IN CHRONIC BENIGN TERTIAN MALARIA.

BY

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(*Quinine and Malaria Enquiry, Malaria Survey of India, Kasauli*)

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AN investigation was carried out to see what effect, if any, the oral administration of sugar had on the relapse rate of malaria patients when given daily during treatment and during eight weeks' observation after the end of all treatment

## OBSERVATIONS BY OTHER WORKERS

(a) *Blood sugar in relation to blood parasites*—Bass and Johns (1912) showed that dextrose was needed for the growth of the malaria parasite *in vitro* and the former worker also cultivated the parasite from the blood of a diabetic patient without the addition of sugar. This suggests that a condition of hyperglycaemia might be more suitable for the growth of the malaria parasite in the body than the reverse condition.

Sinton and Hughes (1924), however, could trace no relationship between the amount of rise of blood sugar percentage and the number of parasites in the peripheral blood in malignant tertian malaria.

The experiments of Hegner (1926) on bird malaria indicate that the sugar content has a striking effect on the blood as a medium for the growth and multiplication of the malarial organisms of the bird. When canaries were fed on sugar during the daily segmentation period, the parasite number did not reach a peak and then fall as usual, but continued to rise until the birds died and when canaries were treated with insulin to produce a hypoglycaemia the parasite number did not rise as high, on the average, as in control birds. The sugar content of the blood may, therefore, be one of the factors involved in the production of a relapse.

MacDougal (1927) has shown that the course of infections in canaries with bird malaria can be modified by feeding sugar to the birds, thus presumably increasing the sugar content of the blood, and, by injecting insulin into them, thus decreasing the content. Changes in the sugar content of

## CINCHONIDINE

Graph I, fig A, shows the effect produced by 10 mgs of cinchonidine given intravenously. It will be seen that there is a well marked depression of both the auricles and the ventricles, the amplitude and the force of the beat are diminished and the rhythm is slowed.

We next investigated the cause of this depression and slowing. In order to determine if this were due to any effect on the nervous mechanism of the heart, we pithed the animals through the foramen magnum and thoroughly destroyed the medulla and the cerebral cortex by passing a stout seeker inside the cranium. We found this procedure to be more convenient and quicker than the trephine method, as the whole operation including the tracheotomy could be completed within four minutes, the loss of blood was very small, the wound being immediately plugged with strips of gauze. The central connections were completely severed and all the higher centres destroyed. In other experiments we not only destroyed the brain but also the spinal cord by passing a probe into the spinal canal.

Graph I, fig B, shows the effect of injection of 7.5 mgs of cinchonidine in a pithed cat with spinal cord intact. The depression of the amplitude and slowing of rhythm were still present and similar effects were produced when both the brain and the spinal cord were destroyed. This showed that these were not associated with any effect of the alkaloid on the cerebral, medullary or spinal centres.

Graph I, fig C, shows that when the vagi are cut in an animal with brain and the cord intact, cinchonidine still produces its depressing effects. When the terminations of the vagi are paralysed by atropine, this effect remains unaltered (Graph II, fig A). It can, therefore, be deduced from the experiments that stimulation of the inhibitory nervous mechanism is not responsible for the depressing action of cinchonidine on the heart.

We next investigated if the depression of the accelerator nervous mechanism was the cause of the decrease of amplitude and slowing of the rhythm. Graph II, fig B, shows that if the sympathetic ganglia are paralysed by nicotine, injection of cinchonidine still produces the same depressing effects. Paralysis of the sympathetic ganglia, therefore, is not responsible for this action. Graph II, fig C, shows that paralysis of the sympathetic nerve endings with ergotoxin does not interfere with these effects of cinchonidine. Moreover nicotine in small doses and adrenalin are still effective after an injection of cinchonidine. The facts that these sympathetic stimulants are still effective after cinchonidine and this alkaloid still produces its usual effects after the sympathetic is paralysed show that the inhibition of the accelerator mechanism can not be the cause of depression of the heart observed after cinchonidine.

It is interesting to note here that the tone of the vagi is probably depressed rather than stimulated by cinchonidine. That this is so can be demonstrated by the following experiment. In an animal anaesthetised with urethane the vagus is dissected in the neck and is stimulated by induced current from the secondary coil of a Du Bois Reymond inductorium. The minimum stimulus required just to inhibit the heart was determined and the distance of the secondary coil noted.

(c) *Effect of malaria treatment on blood sugar*—Hughes (1925) confirmed the fact that large doses of quinine administered to rabbits produce a rise in the blood sugar. He explains that quinine influences the level of the circulating glucose in two ways, one by stimulating the sympathetic system and so probably causing a secretion of epinephrine, leading to hyperglycæmia, and the other by causing a liberation of insulin with resultant hypoglycæmia. This author (Hughes, 1926) also found that quinine in anti-malarial doses causes a lowering of the blood sugar in man, and this effect can be neutralised by pituitrin injection. Mild symptoms of hypoglycæmia may ensue after quinine administration. These can be prevented by giving carbohydrates or a meal rich in carbohydrates half to one hour before the quinine dose is given.

Hughes and Shrivastava (1927) find that quinine in anti-malarial doses in man causes a retention of phosphates. This fact is evidence of the interference of this drug with carbohydrate metabolism, by causing an increased output of insulin. Therefore, when quinine is administered in larger doses over long periods, as is often done in the treatment of malaria, it is acting more or less continuously as a stimulant of the pancreatic islets.

Mikami (1926) asserts that sodium bicarbonate, sodium carbonate, disodium phosphate, and sodium hydroxide given intravenously may exert a remarkable inhibitory influence on the hyperglycæmia and glycosuria of a rabbit poisoned by a hypodermic injection of CO gas.

These observations suggest that the hypoglycæmia produced by quinine may be one of the causes of the beneficial effects produced by this drug in malaria, while the work of Mikami (1926) may help to explain the good results following the quinine and alkali treatment of Sinton (1926).

#### DISCUSSION OF OBSERVATIONS

These observations seem to indicate that there may be some relationship between the amount of sugar in the blood and the development of malaria parasites in the body.

In most cases the results favour the idea that an increase in the blood sugar synchronises with an increase in the number of parasites, so that the administration of sugar during malarial treatment would favour the development of parasites and hence of a relapse.

Cohn (1927) states, however, that the oral administration of sugar to fever cases did not cause a rise of blood sugar, so possibly such an addition to the diet would have little effect on the number of parasites during the febrile stage of the disease. Sinton and Hughes (1924) found that there was a tendency to liver inefficiency in malaria, so possibly the oral sugar was easily burnt up in combating this. Rudolf and Marsh (1927) record that the administration of glucose during malarial paroxysms caused a relief of the subjective symptoms, and Sinton and Hughes (1924) suggest the administration of sugar to protect the liver during the acute stage of the disease.

The observations would appear to indicate that the oral administration of sugar should have no ill effects during the acute febrile stage of the disease but



# GRAPH II

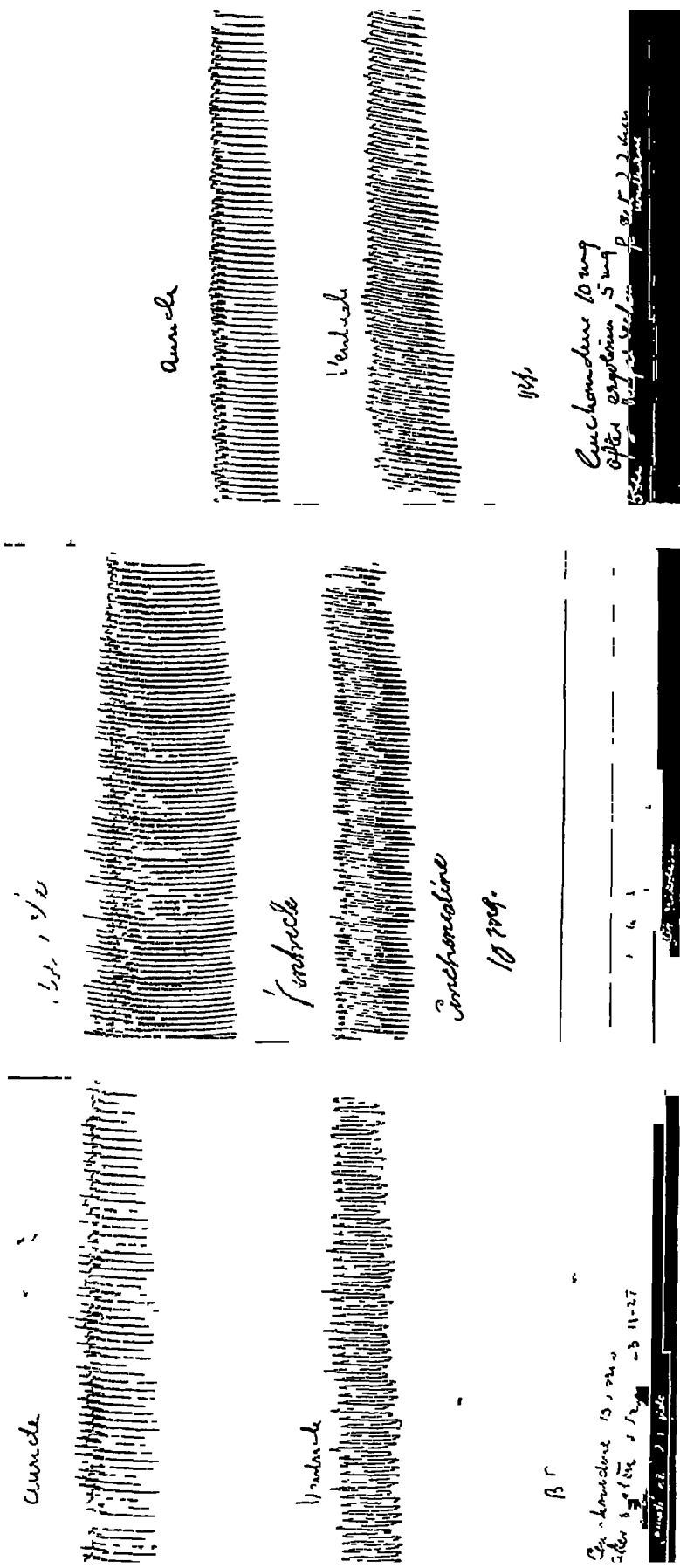


Fig (a)

Cinchonidine after section of the vagi and atropine, usual effect

Fig (b)

(b) Cinchonidine given after nicotine, note slowing and depression

Fig (c)

(c) Cinchonidine after ergotamine and vagal section Slowing and depression are seen

## DISCUSSION OF RESULTS

Stephens, etc (1919), from the observation of 582 relapses due to benign tertian malaria found that 60.6 per cent occurred during the first three weeks after the end of treatment. Acton (1921) also studied the distribution of 978 relapses occurring among cases of benign tertian malaria treated with the cinchona alkaloids. His figures showed that 60 per cent of these occurred during the first three weeks after treatment.

Among the 'sugar series' in this paper about 58 per cent of the relapses occurred before the end of the second week after treatment as compared with 30 per cent among the patients who had not received sugar. In the former series 84.2 per cent of the relapses occurred during the first three weeks after treatment as compared with 60 per cent in the latter series. The figures of the relapses during the three week period among the patients who received no sugar are identical with those of Stephens, etc (1919), and of Acton (1921) while those in the 'sugar series' are much higher.

The relapse rate among those patients who were not given sugar was only 48.8 per cent as compared with a possible maximum of 71.4 per cent and an observed minimum of 67.8 per cent in the sugar series. The relapse rate among the cases which received no sugar is, however, much lower than that usually obtained in this enquiry after quinine treatment and may possibly be due to an error of chance distribution.

These figures, although few in number, appear to indicate that the continued administration of sugar during the treatment and observation of patients given quinine for benign tertian malaria may cause a greater relapse rate but shows that such relapses occur at an earlier date.

The work of MacDougal, Hegner and others goes to show that hypoglycæmia may have a deleterious effect on the development of the malarial parasite in the peripheral blood, and the necessity for dextrose in the cultivation of the malarial parasite *in vitro* points to a condition of hyperglycæmia as being more favourable for this parasite.

During the acute stages of the disease the work of Sinton and Hughes (1924) and of Rudolf and Marsh (1927) would point to sugar administration as being beneficial at this period of the disease. On the other hand the results recorded above indicate that the prolonged use of such a diet may be provocative of relapse, and further work should be done to confirm or disprove this conclusion.

## CONCLUSIONS

Although the number of patients observed is comparatively small, the results point to the conclusion that after the febrile stage has passed the prolonged administration of sugar is contra-indicated during quinine treatment. It is possible that a diet low in carbohydrate might be more suitable under these conditions.

## ACKNOWLEDGMENTS

My thanks are due to the Director of Medical Services in India for the facilities which he has placed at my disposal for carrying out this work, and for permission to publish this paper.

soon as the circuit was made. The magnet was taken in the primary circuit and the surface of the heart was stimulated by platinum electrodes from the secondary coil. Graph III, fig. B, shows that the refractory period is considerably prolonged in a turtle's heart. Before the injection, a stimulus applied anywhere in the diastolic phase produced a contraction and a compensatory pause. After an injection of 15 mg of cinchonidine, however, the refractory period was prolonged far into the diastolic phase so that stimuli applied during 9/10 of diastole were ineffective. The heart only responded to stimuli applied late in diastole.

Graph III, fig. C, shows the effects on the refractory period of the mammalian heart. It will be noted that, while before the injection of cinchonidine, the stimuli were effective at the beginning of the diastole, after the injection the stimuli only became effective when they were applied much later in diastole. With large doses the refractory period is prolonged to well beyond the middle of diastole.

The marked prolongation of the refractory phase of the heart led us to investigate the action of the drug in cardiac irregularities. Aconitine in small doses produces an irregularity of the heart by its stimulant action on the vagus centre in the medulla and its power of increasing the irritability of the myocardium. Graph VII, fig. B, shows the marked irregularity produced in the auricles and ventricles after an intravenous injection of 0.04 mg of aconitine. The condition of the heart was such that under ordinary circumstances there was no possibility of the heart resuming its regularity. At the point indicated, an intravenous injection of 10 mgs of cinchonidine was given. The rhythm was restored to normal. The effect, however, was not permanent, the heart in this case regaining its irregularity after an interval of about 7 minutes.

The restoration of the rhythm was due to the lessened irritability of the heart muscle and prolongation of the refractory phase which allows the extra impulses to pass off without eliciting any contraction. Possibly the depression of the vagus mechanism is also responsible for the beneficial action observed in this kind of irregularity.

Isolated strips of turtle's heart free from nerve endings were prepared and suspended in oxygenated Ringer's solution. The addition of the cinchonidine to the perfusate in concentration of 1-8,000 produced slowing of rhythm and weakening of the individual contraction. This further shows that cinchonidine has a direct action on the heart muscle.

Perfusion of the mammalian heart by Langendorff's method also shows the depression of the heart in dilutions of 1-100,000 to 1-20,000 and less.

The lessening of the amplitude, dilatation of the heart, diminution of excitability, prolongation of the latent period and refractory periods and behaviour of isolated strips all support the view that cinchonidine acts directly on the heart muscle.

#### CINCHONINE

When cinchonine is given intravenously in 5 to 10 mg doses it produces a marked increase in the amplitude of the auricular beats and a very slight decrease

# GYMNEMA SYLVESTRE IN DIABETES MELLITUS

BY

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*Gymnema sylvestre* is a stout, large, woody, climbing plant which grows abundantly in Central and Southern India and is also distributed to the Tropical Africa. It is known in Sanskrit as 'Meshasringi' meaning ram's horn, in Hindi it is called 'Merasingi,' in Bengali 'Chota-dudhilata,' and in Bombay 'Kavali' or 'Vakandi'. Edgeworth noticed that when leaves of this plant were chewed the power of the tongue to appreciate the taste of sugar and all saccharine substances was abolished. This was confirmed later by Hooper who discovered that the leaf also had the valuable property of completely removing the taste of bitter articles such as quinine. The loss of these sensations does not last for 24 hours as was stated by Edgeworth, but only from one to two hours. The root of the plant has a reputation among the Hindu physicians as a remedy for snake-bite. The powdered root is generally applied locally to the part bitten and a decoction is administered internally.

On account of its property of abolishing the taste of sugar it has been given the name of 'Gur-mar' meaning 'sugar destroying,' and the idea has gained ground in some quarters that it might neutralise the excess of sugar present in the body in diabetes mellitus. In Bombay and Central India it has been used as a remedy against this condition and wonderful results have been claimed. The present investigation was undertaken to see if the drug really had any effect on the blood sugar in man and animals.

## CHEMICAL COMPOSITION

Hooper (1887) made a systematic examination of the leaves. The powdered leaves were extracted with various solvents and the residue from the alcoholic

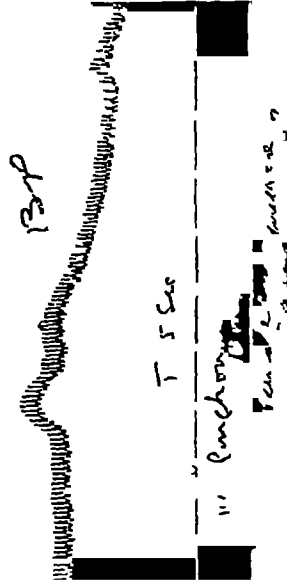
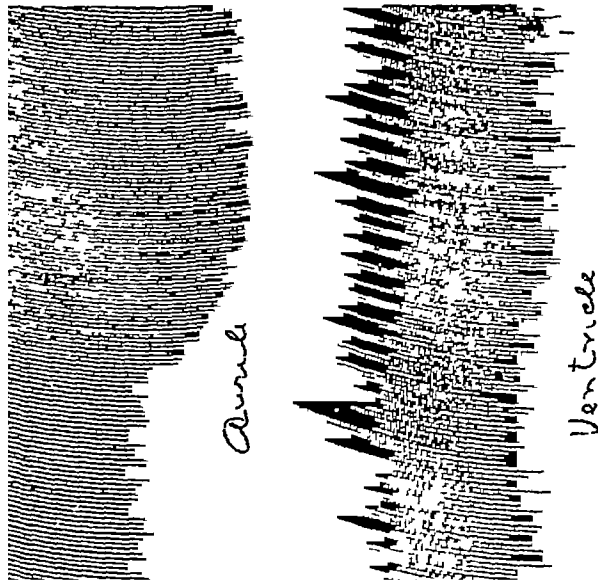


Fig (a)  
Note the stimulation of the auricle and acceleration of rhythm produced by cinchonine

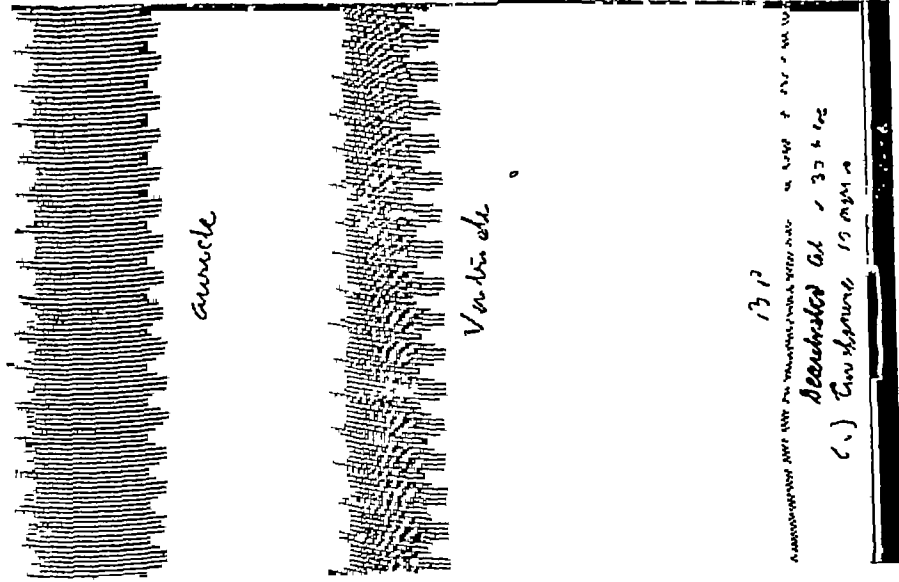


Fig (b)  
Stimulation of the auricle not seen in decerebrated cat after injection of cinchonine

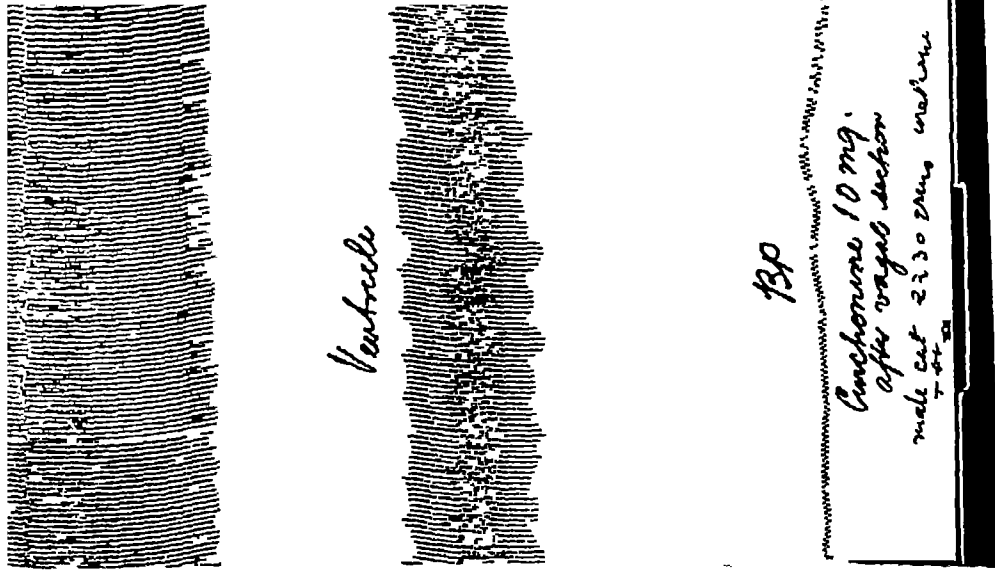


Fig (c)  
Stimulation of the auricle absent after vagal section

Fodar and Reifenberg have shown that green macerated tobacco leaves contain peroxidase, oxidase and catalase and the dried leaves peptidase, peroxidase, catalase, and an enzyme which breaks down nicotine to organic bases and ammonia. Similar enzymes have also been shown to exist in digitalis leaves and other plants. In order to see if the leaves of *Gymnema sylvestre* contained any bodies of the nature of enzymes, the dried leaves were thoroughly extracted with water and filtered. The enzymes were precipitated with 95 per cent alcohol, and purified by redissolving in water and reprecipitating. The final product was washed with absolute alcohol and dried *in vacuo*.

#### EXPERIMENTAL

The action of the enzymes isolated from *Gymnema sylvestre* was studied *in vitro* on both cane sugar and glucose. The sugar solutions were made up to a definite strength, and then mixed with the powdered leaves of *Gymnema sylvestre* as also the enzyme isolated from the leaves. The mixtures were kept in an incubator at 37°C for 48 hours and estimations were made at regular intervals to see if any changes occurred. The following results were obtained —

(A) The reducing substance present in the leaves was found to be 0.37 per cent.

(B) In the cane sugar solution mixed with the powdered leaves, hydrolytic action commenced within 2 hours and was completed in 18 hours. The same result was obtained in the cane sugar solution mixed with enzyme isolated from the leaves.

(C) The powdered leaves of *Gymnema sylvestre* were found to have an oxidase action on glucose solution and glycolysis occurred which reduced the strength of the glucose solution from 2.5 to 0.66 per cent in 29 hours. In the enzyme isolated from the leaves, no such action was seen.

(D) The gymnemic acid was found to have neither hydrolytic nor oxidase action when mixed with cane sugar or glucose solution.

The effect of the drug on the blood sugar was tested on rabbits. The animals used were carefully selected, were all over 1.0 kilogram in weight, and were of the albino Himalayan and the brown Belgian hare types. A quantitative estimation of the initial blood sugar was made and then the drug was given by subcutaneous injection. Two hours after the injection the blood sugar was re-examined. Besides pure gymnemic acid the following fractions were tried and the effect on the blood sugar recorded.

- (1) An aqueous extract of the powdered leaves
- (2) An alcoholic extract using 95 per cent alcohol
- (3) An alcoholic extract using 70 per cent alcohol
- (4) Sodium salt of gymnemic acid

The results of these experiments are given in Tables I, II, III, IV and V.

A perusal of the tables shows that in none of the animals was there any reduction in the amount of sugar present in the blood. It may be argued that the non-reduction of blood sugar in these rabbits after injection of the various preparations of *Gymnema sylvestre* might be due to the excess of glycogen in the liver of these rabbits, preventing the fall in blood sugar by tending to raise it.

Graph VI, fig B, shows that the refractory period is not appreciably affected in the mammalian heart, stimulation of the surface of the ventricle just at the beginning of diastole being still effective after large doses of cinchonine. In the turtle's heart, smaller doses do not alter the refractory period at all but larger doses increase it so that stimuli applied during the first third of the diastolic phase do not elicit any contraction (*see* Graph VI, figs C, D and E). The prolongation, however, is not so marked as in the case of cinchonidine.

To study the effect of cinchonine on the irregularities of the heart the same procedure as described in the case of cinchonidine was followed. Graph VII, fig A, shows the irregularity produced in the auricle and ventricle of a cat by 0.04 mg of aconitine, and the restoration of normal rhythm by an injection of 10 mgs of cinchonine. The drug owes its action to the depression of the vagus which is markedly stimulated by aconitine. The effect in this case, as with cinchonidine, was not very lasting.

From these experimental data we are justified in concluding that cinchonine produces a stimulation of the auricle by its depressing effect on the vagal centre in the medulla and depresses the ventricle by its direct action on the muscle fibres. Our results confirm the views held by Clerc and Pezzi that cinchonine, at any rate, depresses the vagal centre in the medulla. We have also produced experimental evidence to show that the vaso-motor centre is also depressed, as the fall of blood pressure after injection of cinchonine is not nearly so marked in the case of decerebrated animals as when the medullary centres are intact.

### DISCUSSION

From the experimental evidence produced above it will be seen that the two dextro-rotatory alkaloids of cinchona bark, quinidine and cinchonine, have a very similar action on the heart. They both produce an apparent stimulation of the auricle by their depressant action on the vagal centre in the medulla and depression of the ventricle by their action on the myocardium.

Depression of the vagus centre should produce a stimulation of the auricle as well as of the ventricle. After cinchonine, however, the auricle alone is stimulated. Similarly the toxic action of the drug should manifest its effects on the auricular as well as ventricular muscle, while actually only the ventricle is depressed. These results are difficult to interpret but the possible explanation might be that although both the factors are operating in each case, in the case of the auricles the nervous factor predominates while in the case of the ventricles the direct toxic effect of the drug on the muscle fibres overbalances the nervous effect. Starling has pointed out that it is doubtful if the vagus has any direct action on the mammalian ventricle, its effect at any rate is slight as compared with that on the venous end of the heart. According to Leatham, the ventricular muscle of amphibia contains sympathetic nerve endings but no vagal endings. Depression of the vagal mechanism, therefore, produces the physiological effects mostly on the auricle. Cinchonine produces slight depression of the auricle instead of stimulation, if the termination of the vagi are paralysed by atropine. Cinchonidine has a much more toxic effect on the muscle so that even though it

TABLE III

*Action of the Alcoholic extract (70 per cent alcohol) of Gymnema sylvestre on rabbits*

(Strength 1 c c = 1 gramme of leaves )

Date	Identification No of rabbit	Dose injected	Blood sugar before	Blood sugar 2 hours after
			Per cent	Per cent
20-4-27	Brown—5	1 c c	0 072	0 065
	Brown—7	2 c c	0 084	0 080

TABLE IV

*Action of Pure Gymnemic acid on the blood-sugar of rabbits*

(Strength 0 1 gramme in 1 c c )

Date	Identification No of rabbit	Dose injected	Blood sugar before	Blood sugar 2 hours after
			Per cent	Per cent
12-5-27	Brown—9	$\frac{1}{2}$ c c	0 084	0 080
	Brown—10	1 c c	0 084	0 070

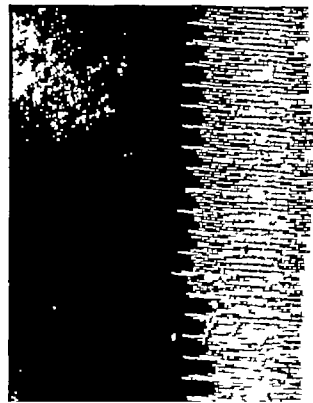
TABLE V

*Action of the Sodium salt of Gymnemic acid on the blood-sugar of rabbits*

(Strength 0 1 gramme in 1 c c )

Date	Identification No of rabbit	Dose injected	Blood-sugar before	Blood sugar 2 hours after
			Per cent	Per cent
23-6-27	Brown—12	2 c c	0 083	0 096
30-6-27	Brown—16	3 c c	0 096	0 102
	Brown—17	2 c c	0 102	0 100
9-8-27	Brown—19	2 c c	0 095	0 110
	Brown—20	2 c c	0 105	0 098
	Brown—21	3 c c	0 115	0 090
20-12-27	Brown—22	3 c c	0 102	0 100
	Brown—23	2 c c	0 114	0 118





Cardiometer

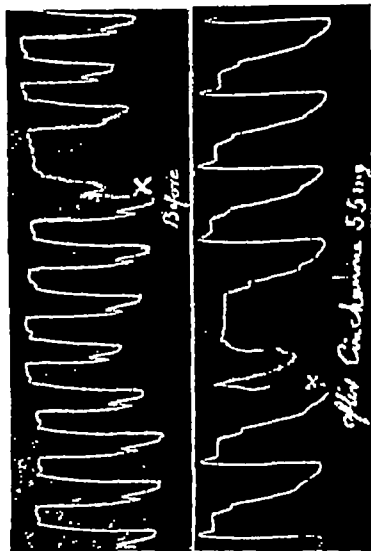


Fig (b)

(b) Mammalian heart refractory period, up stroke diastole Upper tracing normal heart Lower tracing after 55 mg of cinchonine No alteration seen

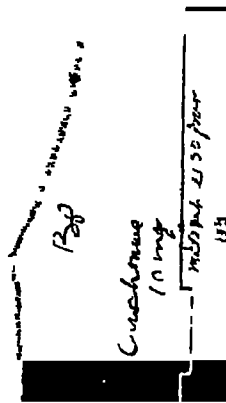


Fig (a)

Cardiometer tracing Note the slight stimulation

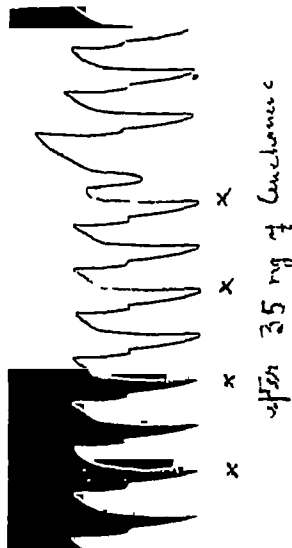


Fig (d)

(d) Refractory period of turtle's heart after 35 mg of cinchonine Note stimuli ineffective when applied during the first 1/3rd of diastolic phase They are only effective when applied about the middle of diastole

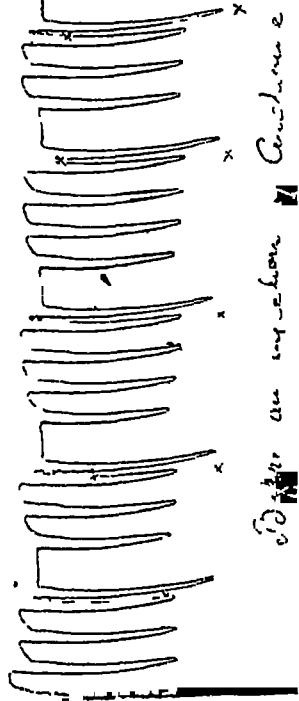


Fig (c)

(c) Refractory period of turtle's heart normal tracing up stroke diastole, stimulus at X Note that stimulus effective anywhere in diastole.

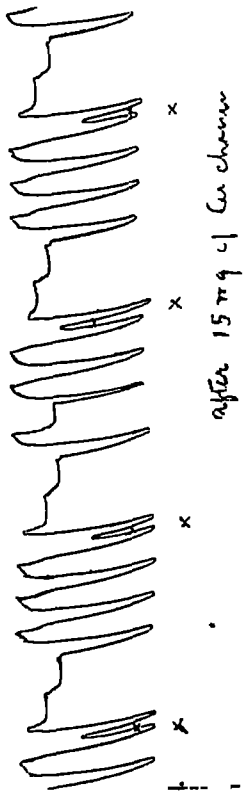


Fig (e)

(e) Refractory period of turtle's heart, after 15 mg of cinchonine. Note stimuli effective anywhere in diastole.

## CASE No 2

Name—Biswas

D Y No 2

Date	Volume of urine in c cs	Sugar per cent	Total sugar excretion in gms	Blood-sugar per cent	Sugar value in diet in gms	Body weight in lbs	REMARKS
12-1-27	1,200	2.5	30	0.129	208	101	Powdered leaves of <i>Gymnema sylvestre</i> , one teaspoonful three times a day
13-1-27	1,200	2.5	30				
14-1-27	1,260	2.4	30.2				
15-1-27	960	3.4	30.6				
17-1-27	1,080	3	32.4			101½	
18-1-27	1,200	2.6	31.2	0.121			
19-1-27	1,200	1.9	22.8				
20-1-27	1,200	2.4	28.8				
21-1-27	1,140	2.5	28.5			102	
22-1-27	1,140	2.4	27.3				
24-1-27	1,200	2	24	0.123			
25-1-27	1,200	2	24				

## CASE No 3

Name—M M K

D Y No 4

Date	Volume of urine in c cs	Sugar per cent	Total sugar excretion in gms	Blood-sugar per cent	Sugar value in diet in gms	Body weight in lbs	REMARKS
18-1-27	1,380	5.1	70.3	0.261	246	80	Powdered leaves of <i>Gymnema sylvestre</i> , one teaspoonful three times a day
19-1-27	2,040	3.6	73.4				
20-1-27	2,100						
21-1-27	1,920	4	76				
22-1-27	2,340	3.3	77.2				
24-1-27	2,040	3.3	67.2			79½	
25-1-27	1,500	4	60				
26-1-27	1,500	4	60				
27-1-27	1,440	4.3	61.9				
28-1-27	1,650	3.3	53.4				
29-1-27	1,800	3.3	59.4				
31-1-27	1,140	4.8	54.7	0.240			
2-2-27	1,740	4.6	81				
3-2-27	2,040						
4-2-27	1,500	4.1	61.5			81½	
5-2-27	1,200	5	60	0.246			
8-2-27	1,500	4.5	67.5			79½	
9-2-27	1,500	4.8	72	0.246			



## CASE No 6

Name—N Maharana

K C B 14

Date	Volume of urine in c cs	Sugar per cent	Total sugar excretion in gms	Blood-sugar per cent	Sugar value in diet n gms	Body weight in lbs	REMARKS
11-3-27	1,800	5.3	95.4	0.164	450	120	Alcoholic extract of powdered leaves of <i>Gymnema sylvestre</i> , equivalent to one teaspoonful of powdered leaves three times a day
12-3-27	1,800	5.5	99				
14-3-27	1,320	6.6	87.6				
15-3-27	1,920	5.2	99.5				
16-3-27	2,280	4.3	98	0.209		121½	
17-3-27	2,100	5	105				
19-3-27	2,100	5	105				
21-3-27	1,200	7	84			119	
22-3-27	1,740	4.5	76.6				
23-3-27	2,100	4	84			119	
24-3-27	1,920	5.7	109	0.174		117	
25-3-27	1,800	5	90				
26-3-27	1,620	5.5	89.1				

Case Nos 5 and 6 received same dose of the leaves but in the form of an alcoholic extract

A perusal of the tables will show that the drug had no appreciable effect in reducing either the blood sugar or the total daily output of the urinary sugar. It will be seen that in some cases the total excretion of sugar became slightly less towards the end of the treatment, but such variations may be accounted for by the restricted diet alone. The slight variation in the blood sugar may be accounted for in the same way. Administration of insulin to all these cases rendered them sugar free.

## CONCLUSIONS

From the results of our observation we feel justified in drawing the following conclusions —

(1) The leaves of *Gymnema sylvestre* contain a substance which has a hydrolytic action on cane sugar. There is also an oxidase-like substance which produces glycolysis in a solution containing glucose.



# A COMPARATIVE STUDY OF THE ACTION OF CINCHONIDINE AND CINCHONINE ON THE HEART

BY

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SINCE the introduction of Cinchona bark into therapeutics considerable experimental work has been done on two of its alkaloids, namely, quinine and quinidine, but little attention has been paid to cinchonidine and cinchonine. These latter alkaloids have been passed over with the remark that they have the same action as quinine. Fredricq and Terroine working with cinchona alkaloids on the isolated turtle's heart found that all of them diminished the frequency and amplitude of the heart beat, producing a diastolic arrest, and that lævo-bases were more powerful than their dextro-rotatory isomers. De Arric (1912) found that the turtle's heart was slowed by all doses and the amplitude was decreased by the higher concentrations but increased by the lower ones. McGilchrist (1914) made an extensive experimental study of the cinchona derivatives and strongly advocated the use of cinchonine in benign tertian malarial infections. He made a comparative study of the toxicity of the four chief alkaloids of cinchona bark on guinea-pigs and found that cinchonine was the most toxic and cinchonidine the least. The protozoicidal action of cinchonine, however, was so marked that in spite of its higher toxicity its use against malaria was advocated. Post-mortem examination of guinea-pigs showed that both after cinchonidine and cinchonine the heart was stopped in systole. Biberfeld (1916) found that in mammals and man the cinchona alkaloids quickened the pulse and produced a rise of blood pressure. Cushney described cinchonidine and cinchonine as possessing the same action as quinine and said that the former is more liable to produce symptoms of poisoning. Acton (1922) in his researches on the cinchona alkaloids, pointed out that quinidine and cinchonine were dextro-rotatory while quinine and

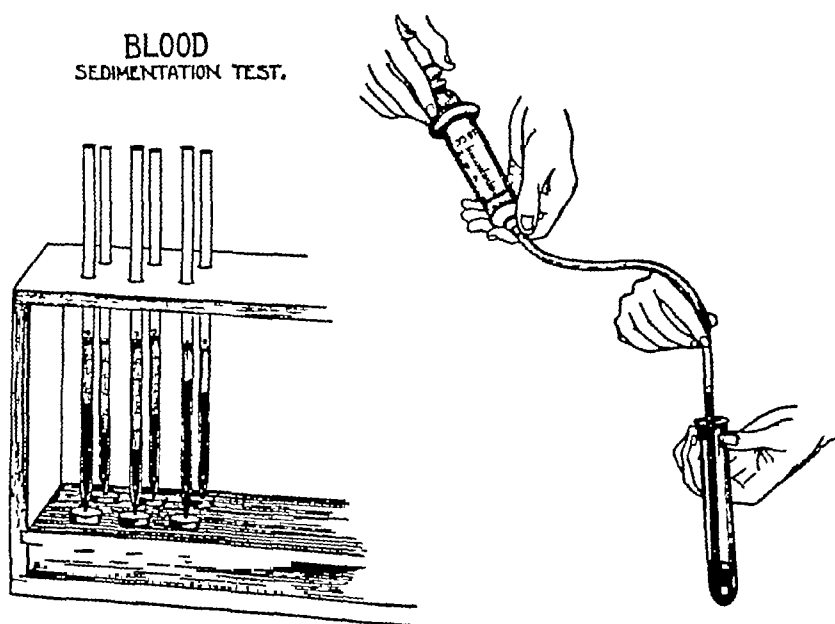
breaking down either of normal tissues or of pathological elements, and consequently setting free of waste material in the body

### EFFECT OF IODIDE ON SEDIMENTATION IN LEPROSY

Potassium iodide has a specific action in breaking down the granuloma caused in the human tissues by the presence of *Mycobacterium lepræ*. This action varies in different stages and types of the disease and it also varies in the same individual at different times apart from increase or diminution of the amount of leproma or without reference to changes in the lepromatous lesions. The effect of this breaking down is generally described as a reaction. The signs of reaction are (a) swelling and vascular engorgement of existing skin and mucous membrane lesions, (b) rise of temperature, (c) the setting free of *M. lepræ* in the blood resulting in cutaneous and subcutaneous emboli indicated by the appearance of small temporary nodules which disappear again in a few days, (d) swelling and tenderness of affected nerve trunks. A more delicate sign of reaction, however, is the sedimentation test which often shows a marked acceleration when other signs of reaction have not yet appeared. This is doubtless due to the fact that iodide breaks down small foci of leproma and the waste material set free produces the same effect that is produced by other waste material in the tissues, that is, it brings about a change in the blood plasma which increases the rate at which the erythrocytes sediment.

### METHOD ADOPTED FOR CARRYING OUT THE TEST

The method used is an adaptation of that used by other workers and is chosen because it makes it possible to test a large number of bloods at once with fair accuracy and with the expenditure of a minimum of time.



0.3 c.c. of a 5 per cent solution of sodium citrate in distilled water is drawn into an all-glass 2 c.c. syringe, 1.2 c.c. of blood is drawn from the patient's vein

*Preparation*—The mother liquor after the separation of quinine is precipitated by ammonia solutions, and the dry precipitate extracted several times by ether, which leaves the greater part of the cinchonine undissolved. The ethereal extract is shaken with dilute HCl, which dissolves the cinchonidine and quinidine. The acid shakings are now exactly neutralised by ammonia solution and to this neutralised solution potassium tartrate is added which precipitates the cinchonidine as an insoluble tartrate. The cinchonidine precipitate is dissolved in dilute acid, and then an excess of ammonia solution added, the precipitate is recrystallised twice from alcohol. The recrystallised alkaloid is now converted into a neutral sulphate, and the sulphate is recrystallised three times by dissolving it in 25 times its weight of boiling water and cooling to 35°C. The crystals which separate at this temperature after the third recrystallisation are generally free from impurities.

*Properties*—Cinchonidine behaves as a diacidic base and yields two series of salts. The neutral sulphate  $B_2H_2SO_4$  forms monoclinic prisms with  $6H_2O$  from cold water or with  $3H_2O$  from hot water, soluble in 1 in 63 at 25°C. The acid sulphate  $BH_2SO_4 \cdot 5H_2O$  is easily soluble in water. The neutral hydrochloride  $BHCl \cdot H_2O$  forms monoclinic double pyramids, or with  $2H_2O$  from saturated aqueous solutions, silky prisms soluble 1 in 38.5 at 10°C. The acid hydrochloride or bihydrochloride forms monoclinic prisms very soluble in water. The tartrate  $B_2H_2C_4H_4O_6 \cdot 2H_2O$  is a crystalline precipitate sparingly soluble in water 1 in 1265 at 10°C or insoluble in the sodium potassium tartrate solution. The melting point of the base is 202.4°C and the optical rotation  $[\alpha] \frac{5^\circ}{D} = -111^\circ$  in alcohol. The salts are not fluorescent in dilute sulphuric acid solutions.

*Tests*—Cinchonidine differs from quinine and quinidine in that it does not give the thalleoquin reaction and is not fluorescent in dilute sulphuric acid solutions, and from cinchonine in being more soluble in ether, in the sparing solubility of its tartrate and in being lævo-rotary.

The alkaloids used in our experiments were specially purified by Mr Nihar Ranjan Chatterjee of the Department of Chemistry, Calcutta School of Tropical Medicine and Hygiene. Our solutions were prepared by dissolving the base in the minimum quantity of N/10 HCl.

## EXPERIMENTS

The experiments were chiefly done on the mammalian heart, cats and rabbits being chiefly used. Turtles and frogs were used to confirm the results obtained from experiments on the mammalian heart. Urethane, supplemented by ether whenever necessary, was used as an anæsthetic. Injections were given through a cannula tied in the femoral vein. Turtles and frogs were pithed and injections were given either intrahepatically or intravenously. For myocardiogram experiments, the right auricle and the right ventricle were gently hooked and attached to heart levers by fine threads, records being taken on a drum moving at medium speed.



above that level by 240 grains of iodide given twice a week over a period of three to six months, even when supplemented by 6 or 7 c c of hydnocarpus oil injected intramuscularly once a week. In some cases, however, it is well to repeat the iodide treatment for a month once a quarter through one or even two years.

2 Not only can this test be used as an index of the length of time during which treatment should be continued, but it is also useful for regulating the amount of dosage and for estimating the patient's tolerance of treatment. The lower the sedimentation index and the more quickly it falls after it has been raised by iodide treatment, the more can treatment be pressed with safety. On the other hand if the non-reaction sedimentation index is high, or if it lingers up after a dose of iodide, care should be taken to improve the general health of the patient and iodide given orally or hydnocarpus injections should be discontinued or should at least not be pressed too much.

3 A third use of the test is in contacts with lepers and cases in which the disease is suspected to exist, but who do not show signs sufficient to make a definite diagnosis. In such cases the sedimentation index may be recorded two or three times at weekly intervals and then tested again after iodide administration. Here, however, care has to be taken as the action of iodide on foci of streptococcal and staphylococcal and other infections will also cause an increase in the index. Such foci will, however, generally show up in the form of follicular or other dermal inflammations when doses of under 60 grains are given. When, however, such signs of septic infection do not appear even with doses of 120 or 240 grains, but when marked acceleration of sedimentation is accompanied by tenderness and swelling of nerve trunks especially the ulnar and peroneals, we have at least strong presumptive evidence in favour of leprosy.

4 The sedimentation test can also be used in testing other drugs regarding their power in breaking down lepromatous tissue. Remedies in leprosy are of two kinds: (1) Those which cause reactions and the breaking down of leproma, and (2) those which stop reactions and raise the general resistance of the patient. We have not yet come across any effective remedy which does not belong to one or other of these groups. To the latter group belong various tonics, adrenaline, and also the heavy metals, antimony, iron, arsenic, copper, etc., when given in small doses, though the heavy metals may cause reactions when given in larger doses. Iodides are by far the most powerful of the former group and the sedimentation test is valuable in testing the relative reaction-producing power of various drugs as compared to iodides. In our experience the most effective treatment is one which produces the maximum breaking down of leproma consistent with the maintenance of the general resistance of the patient. Iodides and other drugs of the reaction-producing group can be pressed with safety when the sedimentation index is low or returns quickly to its former level after having risen in a reaction. When the reverse is the case, however, the reaction-producing agents must be withheld or used with great care and the other group of remedies should be used to improve the general resistance.

The use of iodide in leprosy has been described elsewhere (Muir, 1927 and 1928).

auricle

Ventricle

88

2. Lumen 0 mm

After Vagus section

auricle

Ventricle

88

(6) Lumen 7.5 mm

Recurrent 1st

2. Lumen 1.5 mm

auricle

Ventricle

88

Cinchonidine 5 mg 10 mg

1. Lumen 1.5 mm

2. Lumen 1.5 mm

auricle

Ventricle

88

Cinchonidine 5 mg 10 mg

1. Lumen 1.5 mm

2. Lumen 1.5 mm

(a) Cinchonidine, 10 mg, note marked depression and slowing of the auricle and ventricle.

(b) Decerebrated cat, cinchonidine still produces depression of amplitude and slowing of rhythm

(c) Cinchonidine produces depression and slowing after section of both vagi



An injection of cinchonidine was then given and the minimum stimulus required to produce inhibition of the heart was again determined. A perusal of the following table will show that a stronger stimulus is required to bring about the vagus action, after an injection of cinchonidine than before it.

*Distance in cm. of secondary coil from primary*

No	Before injection	After injection
1	7 cm	5 cm
2	9 cm	6 cm
3	11 cm	5 cm

Cinchonidine, therefore, produces a depressing effect on the heart even though it produces a depression of the vagal mechanism. It will thus be seen that neither the stimulation of the vagal mechanism, nor the inhibition of the accelerators is the cause of the depression of the heart produced by cinchonidine. We feel justified in concluding that the seat of action is directly on the heart muscle. Cinchonidine acts directly on the myocardium and depresses it. In order to confirm our conclusion we tested the various properties of myocardium. The amplitude of individual contractions is already shown to be diminished by an injection of cinchonidine. Graph III, fig A, shows that there is dilatation of the heart, which lends support to the view that the drug inhibits the inherent tone of cardiac muscle.

The irritability of the heart muscle was studied by directly stimulating the surface of the ventricle by induced shocks applied with a bipolar platinum electrode. In one of the experiments before an injection of cinchonidine the mammalian ventricular muscle responded to induced shocks when the secondary coil was 13 cm away from the primary. After cinchonidine was given the coil had to be brought nearer to 9 cm, so that a stronger stimulus had to be used before similar effect was produced. We are justified in concluding that injections of cinchonidine diminishes the irritability of the heart muscle.

The latent period was studied in frogs. A Stannius preparation was made by putting a soft clamp over the white crescent and the ventricle stimulated by minimal induced shocks. The point of stimulation was marked by a signal magnet in the primary circuit which was writing exactly in the same perpendicular line as the heart lever to which the apex was attached. The normal interval between the moment of stimulation and beginning of contraction was measured. The clamp was then removed and the heart allowed to beat normally for about 5 minutes. An injection of cinchonidine was given intrahepatically and the heart allowed to beat for 2 or 3 minutes. The crescent was again clamped, the ventricle stimulated and the interval between stimulation and contraction measured as before. It was found that the latent period was more than doubled after injection of cinchonidine (Graph VIII, fig D).

The refractory period of the heart was studied according to the technique described by Waddell. A straight wire was attached to the armature of a signal magnet and was so arranged as to pull the writing lever away from the drum as

*The patients*—The patients were all in-patients in the Carmichael Hospital for Tropical Diseases, two were Jews, one of each sex, 7 were Anglo-Indians, 2 females and 5 males, and 13 were Indians, 12 females and 1 male

The patients were not in any way selected but were a consecutively admitted series of patients put on treatment as they were diagnosed. The patients were either previously untreated or had received a definite course of injections and had relapsed, if they were of the latter class, they were reported as 'resistant cases'

*The diagnosis*—In every case this was made by demonstrating the presence of the parasite by examination of the peripheral blood, by spleen or liver puncture, or by cultural methods

*Proof of cure*—As in the previous series the subsequent history of the patient was accepted as the final proof of cure, but in every instance a spleen or liver puncture with culture of the material was done before the patient was discharged. In three instances leishmania were grown in the cultures, two of these were the cases classed as failures and the third was a woman who was clinically cured and who is known to have remained in good health for more than 2 years since discharge

*The dosage*—No very rigid system was adopted. Most of the patients received between 10 and 12 injections, the initial dose was usually 0.1 gramme and the maximum 0.25 gramme. Some of the resistant cases received a much longer course of injections and in one case, in which a cure was eventually obtained, 34 injections were given and in another 38 injections, in neither instance did the patient suffer any ill effects from the prolonged dosage

*Results of treatment*—The immediate results of treatment of the 52 patients was as follows—

Discharged cured	48
Failed to respond to treatment	2
Died during treatment	2
	<hr/>
	52
	<hr/>

*The failure*—The first was an Anglo-Indian male, aged 38, who is said to have had 24 injections of urea-stibamine during a period of 4 months without registering any improvement. He was then admitted to the Carmichael Hospital and was given 20 injections of stibosan, totalling 4.6 grammes, there was still no improvement. He was given 38 injections of aminostiburea, totalling 9.1 grammes, during a period of 108 days, but as there was still no improvement treatment was abandoned. The patient died some months later

The second case was that of an Indian female, she had received no previous treatment. She was given 14 injections, totalling 3.3 grammes, parasites were still present and she showed no improvement clinically. She refused to remain for further treatment

*The deaths*—One patient who was progressing very favourably and whose temperature had fallen to normal suddenly developed meningeal symptoms and

To be used (vertical)



131

*Cinchonidine 5 mg*  
*- 1 c.c. boric acid - 2 c.c. water*

Fig (a)

(a) Cardiometer tracing, note production of dilatation and depression of the heart

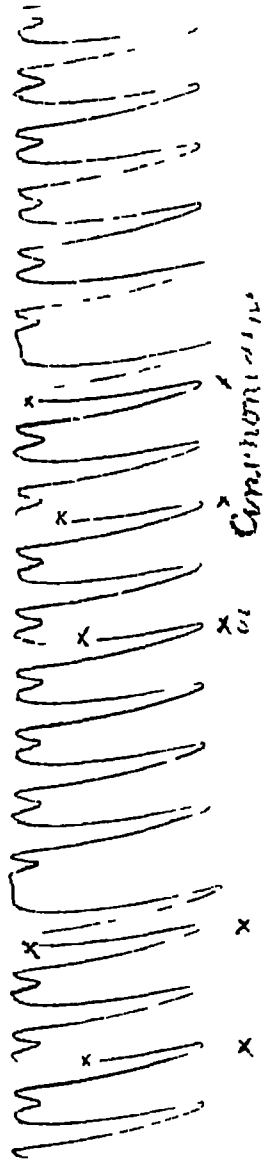
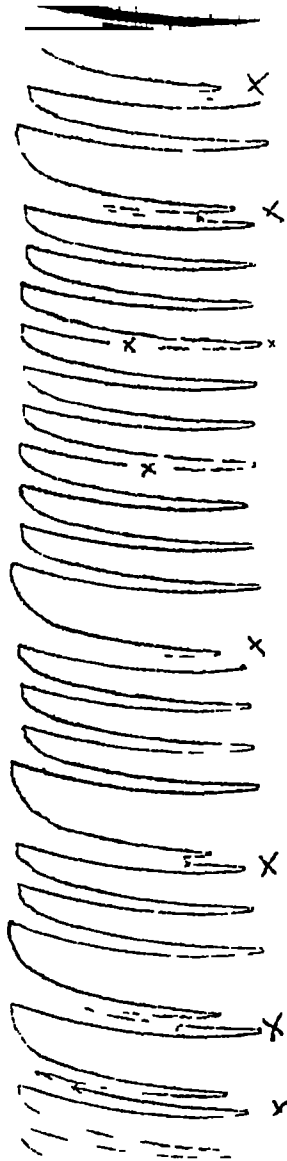


Fig (b)

(b) Turtle's heart up stroke diastole down stroke systole. Stimulus applied at X Upper tracing nor heart, lower tracing after cinchonidine. Note in the upper curve stimuli applied anywhere in diastole are effective while in lower curve they are not effective till applied very late in diastole

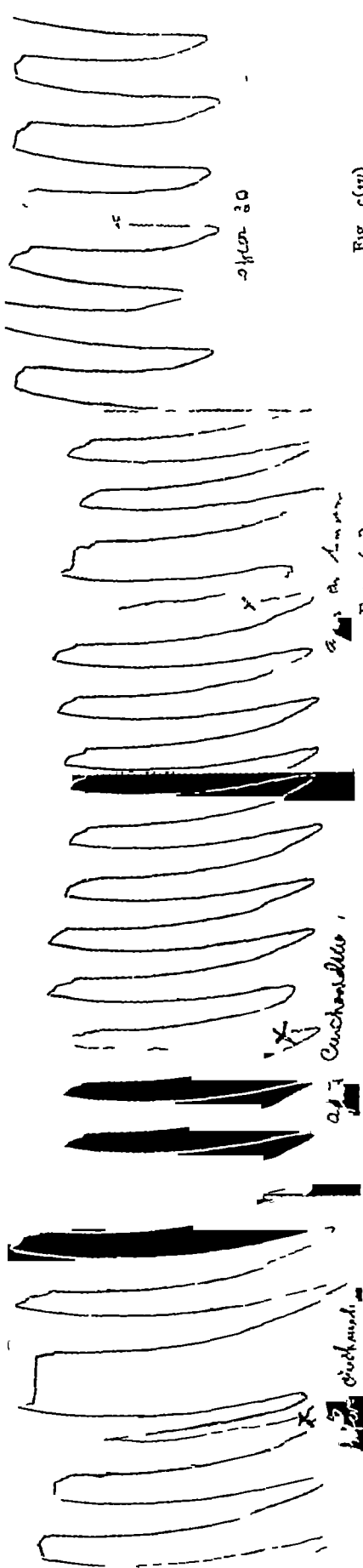


Fig c(ii)

Mammalian heart showing refractory period Up stroke diastole

Fig c(iii)

Stimulus applied at X c(2) shows that in a normal heart a stimulus just at the beginning of

Fig c(iv)

*The number of injections*—The details of the number of injections given in each case are shown below —

Number of injections	Number of ordinary cases in which given	Number of resistant cases in which given.
7	1	
9	2	
10	6	2
11	8	1
12	5	
13	6	
15	2	1
16		1
21	.	2
34		1
	30	8

The mean of the number of injections given in the 30 previously untreated cases was 11·37, and of the 8 resistant cases was 17·25, the mean number given in the whole 38 cases was 12·94

*The actual total dose*—The patients can be divided into groups according to the actual total dose received, as follows —

	Ordinary cases	Resistant cases
Less than 15 grammes	6	
15 to 199 grammes	2	
2 to 249 grammes	9	3
25 to 299 grammes	12	
3 to 349 grammes	1	
35 to 399 grammes		2
40 to 449 grammes		2
Over 50 grammes		1
	30	8

The mean of the total dose given to 38 patients was 2·55 grammes, to the 30 previously untreated patients 2·23 grammes and to the 8 resistant patients 3·7 grammes

in the ventricular beats, the rhythm is somewhat accelerated and the blood pressure falls (Graph IV, fig A) Similar auricular effects have been noticed by Jackson, Friedlander and Lawrence (1922) to be produced by quinidine. The stimulation of the auricle may be either due to the depression of the inhibitory mechanism or stimulation of the accelerator mechanism. In order to study the effect on the inhibitory mechanism the same procedure was followed as described in the case of cinchonidine.

Graph IV, fig B, shows the effect of cinchonine given intravenously in a decerebrated cat. The ventricles are slightly depressed while the auricles are not stimulated. Graph IV, fig C, shows that section of the vagi also abolishes the stimulating effect observed on the auricles. Graph V, figs A and B show that after atropine and nicotine cinchonine does not produce stimulation of the auricle, on the other hand it is quite usual to find a slight but persistent depression after atropine. Failure to produce stimulation of the auricle after nicotine is probably due to the paralysis of the nerve cells in the intracardiac ganglia. The main effect of cinchonine, therefore, appears to be on the cardio-inhibitory centres in the medulla. Direct stimulation of the vagus in the neck shows that a stronger stimulus is required to bring about slowing and inhibition of the heart, after an injection of cinchonine than before it. The drug, therefore, decreases the tone of the vagus and thus may partly be responsible for the stimulating effect observed on the auricles.

Graph V, fig C, shows that paralysis of the sympathetic terminals by ergotoxine does not modify the action of cinchonine on the auricle. Stimulation of the sympathetics, therefore, is not responsible for the auricular effect.

The ventricles are uniformly depressed but to a much lesser degree than in the case of cinchonidine. This is due to the direct toxic action of the drug on the musculature of the heart. Perfusion experiments show that the alkaloid has a depressing action on the musculature of the ventricles in concentrations varying from 1 in 50,000 to 1 in 20,000. The automatic movements of isolated nerveless strips of turtle's heart are diminished with strengths such as 1 in 8,000 but higher dilutions have no effect. Withdrawal of the drug restores the original amplitude of the strips. Graph VI, fig A, shows the effect of cinchonine in cardiometer experiments. It will be seen that whereas in the case of cinchonidine there is dilatation of the heart with weakening of the amplitude, in the case of cinchonine the amplitude is increased and the dilatation effect is comparatively small.

Experiments on the mammalian heart show that injections of cinchonine diminish the irritability of the heart muscle but the effect is not so marked and lasting as that of cinchonidine.

The latent period of the frog's cardiac muscle is not appreciably increased by cinchonine. The same technique as described in the case of cinchonidine was followed. Reference to Graph VII, fig C, shows that even after 1.3 mg of cinchonine given intrahepatically there was no marked prolongation of the interval between the moment of stimulation and beginning of contraction. The period is increased to a very slight extent.



The mean actual total dose of the previously untreated cases, including the two doubtful relapses and the one failure, was 2 30 grammes, and the mean total relative dose 3 31 grammes, that is to say in a mixed population the expenditure of 2 30 grammes per person, or 3 31 grammes per 100 lbs body-weight of patient, should produce a cure-rate of at least 90 9 per cent

*Complications and sequelæ*—Seven patients suffered from jaundice within two months of being discharged as cured. In most instances the jaundice was of short duration but in one case it persisted for about 2 months

Two patients suffered from transient swelling of the face and vomiting after the 6th and 10th injections, respectively, in the former case tolerance was established by lowering the dose and then increasing it slowly but slight swelling followed some of the subsequent injections, and in the latter case the injections were discontinued

*The resistant cases*—Amongst the 38 patients who are known to have been cured, 8 patients had previously received at least one full course of treatment and had either shown no improvement or had relapsed after a temporary improvement. As some of these patients had shown an unusual degree of resistance to treatment, a few details of the individual cases are given

I B, Hindu female, aged 35. She had previously received 30 injections of sodium antimony tartrate, a 'full course' of urea-stibamine (Brahmachari) and a 'full course' of stibosan. She showed only temporary improvement after each course, she had received no antimony injections for 2 months prior to her admission. She was given 34 injections of aminostiburea, totalling 7 2 grammes, and is known to have remained in good health for two years

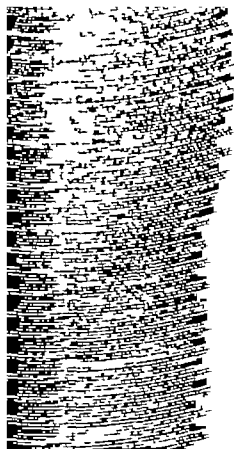
K A, Mohamedan male, aged 36. He had received three courses of injections, 40 injections of sodium antimony tartrate, 18 of stibosan and 8 of urea-stibamine. He had shown only slight temporary improvement after each course. He was completely cured after a course of 15 injections of aminostiburea

S R., Indian Christian boy, aged 10. He had been given two 'full courses' of injections of sodium antimony tartrate of 40 injections each without showing any improvement, and he only showed temporary improvement after a 'full course' of urea-stibamine. He had received no antimony injections for 3 months prior to admission to hospital. He was cured by a course of 21 injections of aminostiburea

Of the other five resistant patients one had previously received a course of 12 injections of stibosan and the other four a course of injections of sodium antimony tartrate

### CONCLUSIONS

Aminostiburea is a drug of very considerable value in the treatment of kala-azar. It is a drug with a low relative toxicity to mice and is well tolerated by man in comparatively large doses



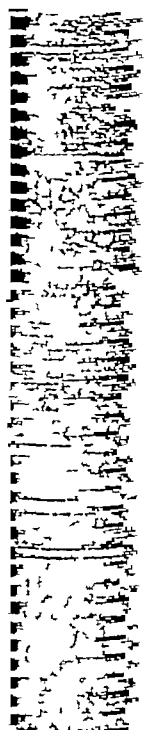
auricle



auricle



auricle



auricle

828

Amphetamine 75 mgms

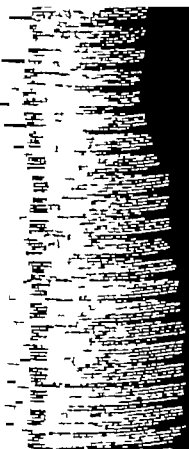
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auricle

Amphetamine 75 mgms

1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22-23-24-25-26-27-28-29-30-31-32-33-34-35-36-37-38-39-40-41-42-43-44-45-46-47-48-49-50-51-52-53-54-55-56-57-58-59-60-61-62-63-64-65-66-67-68-69-70-71-72-73-74-75-76-77-78-79-80-81-82-83-84-85-86-87-88-89-90-91-92-93-94-95-96-97-98-99-100



auricle

828

Amphetamine 10 mgms

1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22-23-24-25-26-27-28-29-30-31-32-33-34-35-36-37-38-39-40-41-42-43-44-45-46-47-48-49-50-51-52-53-54-55-56-57-58-59-60-61-62-63-64-65-66-67-68-69-70-71-72-73-74-75-76-77-78-79-80-81-82-83-84-85-86-87-88-89-90-91-92-93-94-95-96-97-98-99-100

Fig (a)

Fig (b)

Fig (c)

(a) & (b) Note that after atropine or nicotine the stimulation of auricles is not observed, after atropine there is a definite depression of the auricle

(c) After ergotamine the stimulation of the auricle is present



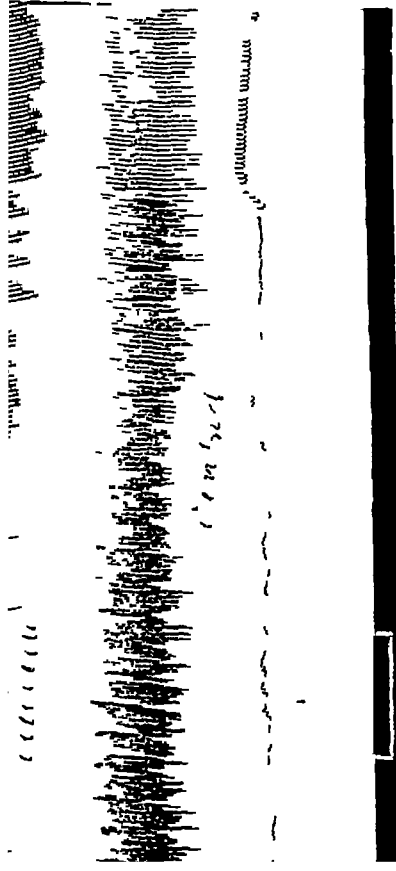


Fig (a)  
 (a) The irregularity of the auricle (upper tracing) and ventricle (lower tracing) produced by aconitine is corrected by cinchonine. Note improved blood pressure

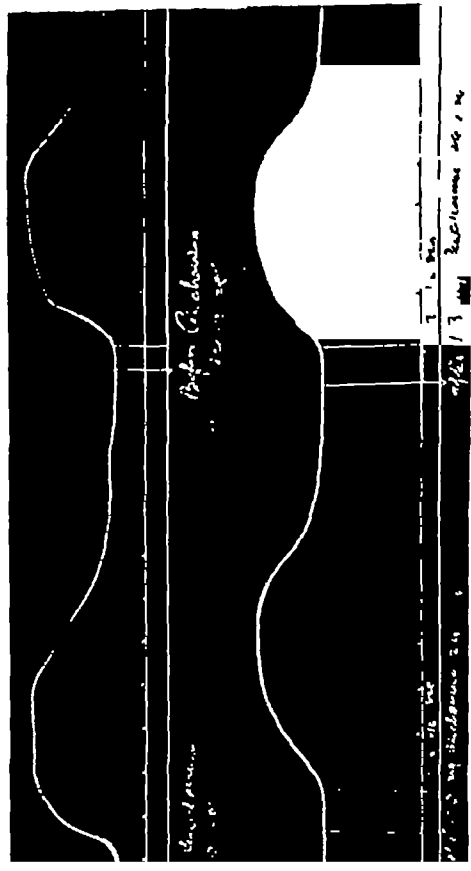


Fig (c)  
 (c) Study of the latent period. Upper tracing normal curve, lower tracing after 1.3 mg of cinchonine. Note a slight prolongation of the latent period

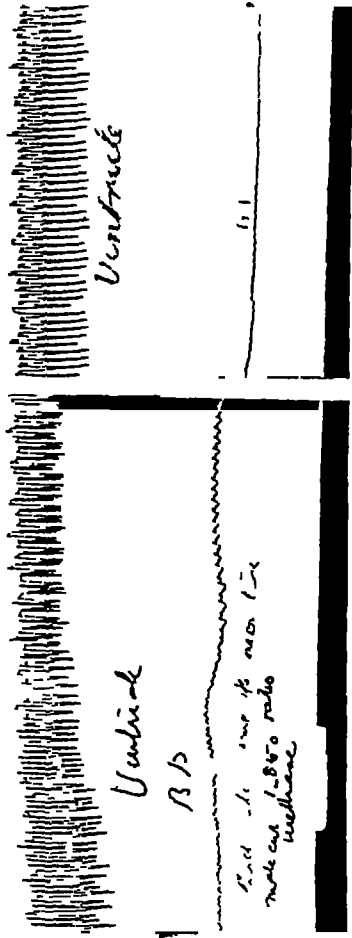


Fig (b)  
 (b) The irregularity of the auricle and ventricle produced by aconitine is corrected by cinchonidine

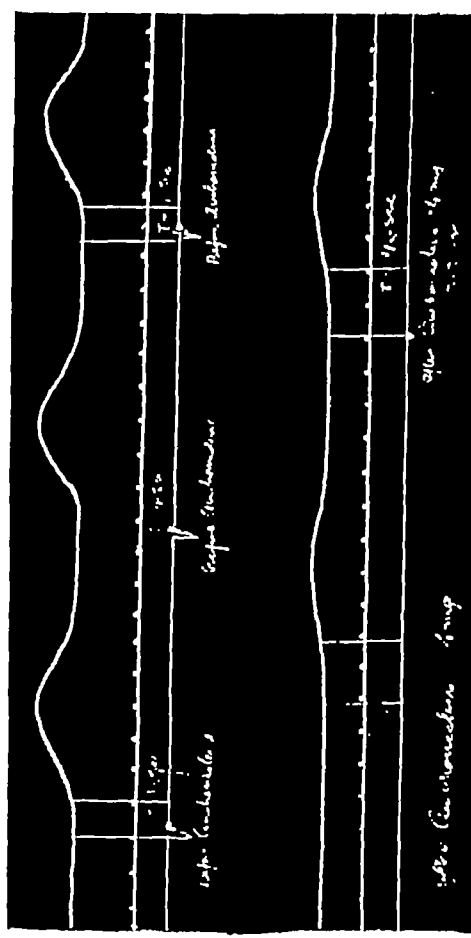


Fig (d)  
 (d) Study of the latent period. Upper tracing normal curve, lower after 0.4 mg of cinchonine. Note that latent period is almost doubled

hospital patients who have been kept under observation for some time after the operation, no ill-effects have ever been noted. The operation has also been performed in the out-patient department apparently without any bad results.

*Description*—The syringe that the writer uses for this operation is a 5 c cm Roux syringe, and the attachment which is described below is made to fit this syringe. By a slight modification in the design it could probably be made to fit almost any type of syringe.

It consists of two oval plates of metal, A and B, held parallel to one another by two metal tie-rods, C, the ends of which are attached at right angles to the metal plates. In one metal plate there is a circular hole and a groove in which the collar of the syringe lies, the latter is held in position by means of three L-shaped bolts, D, which pass through the plate and are secured by nuts (Plate XV, figs 3 and 4).

(A) Oval plate, 5.0 cm  $\times$  3.5 cm  $\times$  0.3 cm. At points 0.1 cm from each end of the long diameter a hole of 0.25 cm diameter is bored through the plate, through these holes the ends of the tie-rods pass and are secured by nuts on the other side. In the centre of the plate a recessed hole is cut, the greater diameter of which is 2.5 cm and the lesser 2.0 cm, the former involving half the thickness of the metal. Grouped round the outside of this hole are three small holes, 0.25 cm diameter, through which pass the three L-shaped locking bolts. The hole is to allow the head of the plunger rod to pass through the recess to accommodate the collar, and the three locking bolts can be turned so as to hold the collar of the syringe firmly in position (Plate XV, fig 5).

(B) Oval plate, the same shape as A but only 0.2 cm in thickness. It has the same two holes for the tie-rods but in this case there is a bush so that the rods can be screwed directly into the plate.

(C) Tie-rod, over all the rod is 9.8 cm long, the main portion of the rod is 9 cm long and 0.4 cm in diameter. At either end there are small portions 0.2 cm and 0.6 cm, respectively, which are reduced to 0.25 cm diameter and have a thread, one end of each rod is screwed into plate B, and the other end goes through the hole in plate A and is secured by a nut.

*Comment*—There are many modifications which might be suggested. It is, for example, unnecessarily heavy, but it is very essential that it should be rigid. The metal plate B need not be oval, in fact it would lie more comfortably in the palm if it were an oblong piece of metal about 1 cm broad. An operator with a small hand might find this syringe difficult to hold, the writer, however, has become accustomed to the syringe in its present form and would hesitate to alter its design.

The syringe with its attachment is useful for all blood-withdrawing operations, a free hand is very useful for controlling the patient's arm or causing congestion of the veins.

depresses the vagus, the effects are more than compensated by the powerful depression of the heart muscle

# SUMMARY AND CONCLUSIONS

(1) Intravenous injections of cinchonidine in animals produce a marked depression of the amplitude of auricular and ventricular contractions accompanied by a fall of blood pressure. The irritability of the myocardium is markedly decreased, the latent period and the refractory period are both lengthened.

(2) Cinchonine produces an apparent stimulation of the auricle and a depression of the ventricle. The former effect is shown to be produced by depression of the inhibitory nervous mechanism and the latter by direct action on the heart muscle. The irritability of myocardium is decreased but not to the same extent as with cinchonidine. The latent period is not increased in frog's heart muscle and the refractory period is not appreciably affected.

(3) Both cinchonine and cinchonidine temporarily restore the normal rhythm of the heart after it is experimentally made irregular with aconitine. This is partly due to the depression of the vagal mechanism and partly to direct depression of the heart muscle.

(4) Our experiments show that the lævo-rotatory alkaloid cinchonidine has a more powerful depressant action on the heart muscle than the dextro-rotatory cinchonine.

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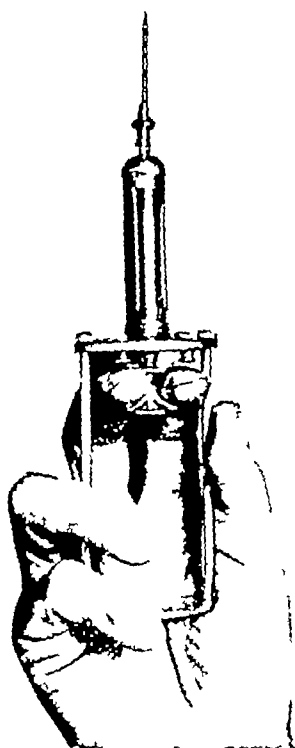


Fig 1

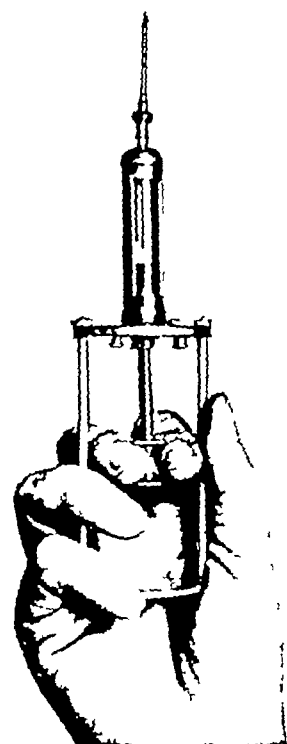


Fig 2

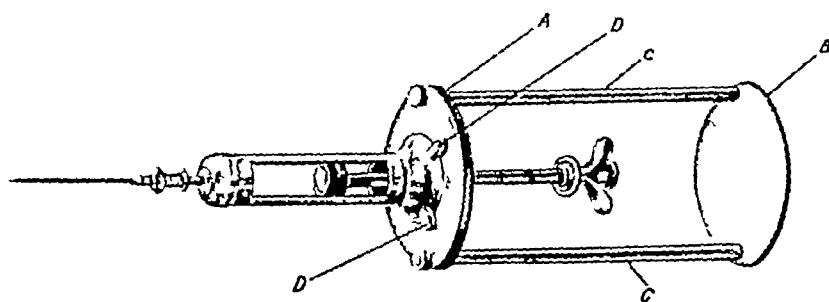


Fig 3

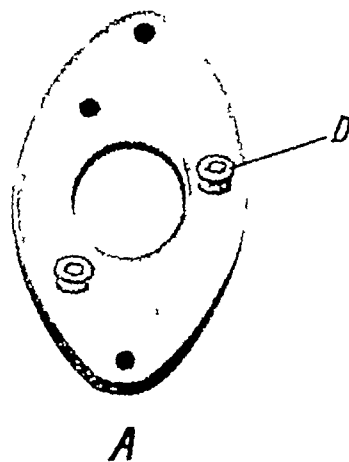


Fig 5

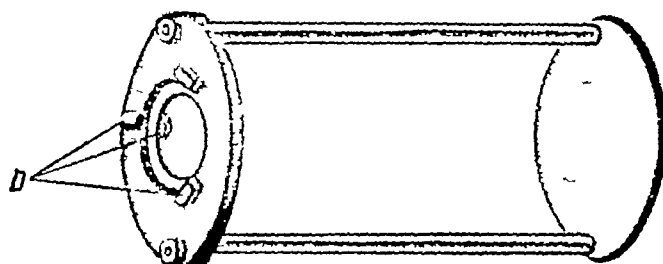


Fig 4

# THE IODIDE-SEDIMENTATION TEST IN LEPROSY

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THE above name is applied to this test because of the information which is gathered from observation of the rate of sedimentation of the erythrocytes of blood taken from leprous patients after administering potassium iodide to them orally

## SHORT HISTORY OF THE SEDIMENTATION TEST

Pribram and Klein (1923) found that the speed of sedimentation of erythrocytes was increased by the following conditions fevers, malignant growths, decrease of total albumen content or of the number of erythrocytes in the blood, decreased viscosity, cholesterol content and content of albumen end-products, while it was retarded in increased albumen content, polycythæmia, hypercholesterolæmia and cyanosis. It is also well known that any condition which causes an excess of bile in the blood retards sedimentation very markedly.

Dreyfuss and Hecht (1922) and others have shown that, though the sedimentation test is useless in the diagnosis of tuberculosis, it is more useful in the diagnosis of the activity of tuberculosis than the observation of the temperature chart.

Puxeddu (1924) showed that the sedimentation of the blood of lepers was accelerated, and was still more rapid when leprosy was complicated by malaria. He showed that this acceleration was due to changes in the serum of the patients and not in the red cells. The opinion of all who have worked on this subject is that the velocity of sedimentation is increased in leprosy—much in nodular or skin cases, less in mixed cases and least in nerve cases—but no attempt has been made so far to use the sedimentation test as a guide to treatment.

It has been agreed that the acceleration of the sedimentation of erythrocytes is due almost entirely to changes in the plasma and not in the cells. What these changes are is still a matter of doubt but it seems clear that the commonest condition in which acceleration takes place is one in which there has been the



of the animals to be experimented with and a small quantity of plant food commonly found growing in the tank from which the animal was collected. If an animal did not attack the larvæ within the first twelve hours it was discarded in order to avoid the risk of its eating the larvæ through mere starvation. The pond-water in the experimental jars was changed every twenty-four hours. A control was always kept in order to ascertain the rate of mortality of the larvæ in the absence of any alien animal.

### Natural Rate of Mortality

When mosquito larvæ were kept in a jar by themselves, on an average 4 to 6 per cent died in 24 hours, without any apparent cause. The larvæ did not show any signs of true cannibalism, that is to say, they did not attack each other, but they readily ate up any corpses, leaving only their hard head capsules behind.

### Enemies

*Hemiptera*—With the exception of Poisson (1926), who observed a species of *Amsops* (Fam. Notonectidæ) preying on mosquito larvæ, it appears that hardly any investigator has recorded bugs as injuring mosquitoes. The following table, in which I have given the species tried and the number of larvæ that they kill in 24 hours, shows that representatives of almost all the families of aquatic Hemiptera are serious enemies of mosquito larvæ, the chief exceptions being very big forms like the giant bug, *Belostoma indicum*, whose proboscis is probably too thick to pierce a mosquito larva—

Name	Family	Number of larvæ that one individual kills in 24 hours
<i>Ranatra longipes</i> Stål	Nepidæ	34
<i>Laccotrephes griseus</i> Montand	Do	26
<i>Plca</i> sp	Notonectidæ	27
<i>Spharodema annulatum</i> Fabr	Belostomidæ	28
<i>Belostoma indicum</i> Lep and Serv	Do	4
<i>Hydrometra</i> sp	Hydrometridæ	15
<i>Micromecta dione</i> Dist	Corixidæ	23

As stated above, bugs kill mosquito larvæ by piercing their body and sucking out their blood with their fine proboscis. Usually the place of attack is the junction of the thorax and abdomen with the result that the larva gets bent in that region. A bug swimming about with a larva at the tip of its proboscis was quite a common sight in my experimental jars. After its body juice has been sucked out, the larva dead and empty, falls to the bottom of the vessel. Sometimes, however, its whole body is reduced to fragments as a result of the numerous and repeated punctures that it receives from the enemy.

*Coloptera*—Both larvæ and adults of the family Dytiscidæ prey on mosquito larvæ. The dytiscid larva is extremely voracious, it pierces a mosquito

into the same syringe and, a small quantity of air having been taken into the syringe barrel, the blood and citrate solution are thoroughly mixed by reversing the syringe several times and the mixture is evacuated into a clean test tube. If several patients are to be tested, their bloods are taken in a similar manner and placed in labelled test tubes in a rack.

An equal number of 1 c.c. pipettes graduated to 1/100ths of a c.c. are placed in a suitable rack with their points plugged by being inserted in holes made in rubber corks (as in the illustration). One of these pipettes is taken from the rack and its upper end attached to a 10 c.c. syringe by means of a rubber tube. The point is inserted in one of the test tubes and, suction being applied by pulling on the piston of the syringe, one c.c. of the blood-citrate mixture is drawn up into the pipette, which is then replaced in the rack, the point again being inserted in the rubber cork which prevents the mixture escaping. The rubber tube is then disconnected from the pipette. In this way the other pipettes are filled up to the 1 c.c. mark from the other test tubes. The top level of the erythrocytes is read off after 2 hours and again after 3 hours and the average of these two readings is taken as the index of sedimentation.

In describing the stages and types of cases of leprosy the classification adopted at the Calcutta School of Tropical Medicine is used (Muir, 1925 and 1928). The normal reading is 10 to 20, in early leprosy ( $A_1$  cases) it is generally 10 to 20, while in  $B_2$  or  $B_3$  cases it is from 40 to 60 when there is no reaction, and during a reaction it may rise to as much as 80.

#### INFORMATION AFFORDED BY THE IODIDE-SEDIMENTATION TEST

1. In a (nodular)  $B_2$  or  $B_3$  case the index is almost invariably high, apart from reactions caused by administration of iodide or otherwise, varying from say 40 to 60. If a small dose of iodide (say 5 to 20 grains) is administered, the index rises by 10 to 30 points and as a rule falls again to its former level within two or three days. As treatment with iodide proceeds and the patient improves, the non-reaction level gradually falls till it reaches what may be considered as normal, viz., 10 to 20. Reactions caused by iodide may still raise the index to 40, 50 or even higher, but it falls again to the lower level when iodide is discontinued. Subsequently, even the maximum dose of 240 grains administered between 6 and 9 p.m. twice a week, fails to cause any rise in the sedimentation index. In such cases intramuscular injections of 7 or 8 c.c. of creosoted hydnocarpus oil given on the same day as 240 grains of iodide, may still have the effect of raising the index when iodide alone fails. In the end even this combined treatment causes little or no rise. Most cases will have become bacteriologically negative and will have ceased to show any other signs of reaction under iodide long before this point is reached. In other words the iodide sedimentation test continues to indicate the presence of the disease long after all other outward signs have gone, and is therefore helpful in judging how long treatment should continue. We generally make it a rule that treatment may be discontinued when the following conditions are found: (a) the patient has become bacteriologically negative, (b) his sedimentation index has fallen to below 25 and is not raised

ate 42 larvæ during 24 hours, an old one (2.5 in across carapace) attacked only 5

*Mollusca*—Certain molluscs appear to destroy mosquito larvæ in a curious manner. In a one-litre jar, about  $\frac{3}{4}$  full of pond-water, one apple-snail, *Pila globosa* Swainson (Ampullariidæ) and 50 larvæ were introduced one afternoon. Next morning thirty-six dead larvæ were found in the jar. The bottom of the jar was covered with the excreta of the snail, and the water was slightly smelling and had turned turbid and yellow. The experiment was repeated several times with almost similar results. If the larvæ were put in the water in which the snail had been kept for the previous 24 hours, about 25 out of 50 larvæ died in a day. When the molluscs *Acrostoma variabilis* (Benson) form *varicosa* (Melanoidæ), *Vivipara bengalensis* (Lam.) form *typica* (Viviparidæ) were put with mosquito larvæ, for each snail kept 25 to 30 larvæ died in 24 hours. It is interesting to add that the planorbid mollusc, *Indoplanorbis exustus* Deshayes, gave negative results. The rate of mortality in the control of these experiments was about 5 per cent.

Of course, the above-named molluscs do not eat mosquito larvæ. The problem as to how they affect the larvæ is very complex, and requires thorough investigation before one can hope to find its solution. Only some preliminary remarks can be made at this stage. The fact that the larvæ when put in the water previously inhabited by the snails die in large numbers suggests that the latter pollute the water, obviously with their excreta or slime or both. It may be pointed out, however, that the larvæ die in larger numbers when kept with the snail than when kept in the water previously inhabited by the latter. The snail *Pila globosa* is more injurious than the other molluscs named above, probably because, being larger, it produces much more excreta.

To ascertain the nature of this pollution, the oxygen and carbon dioxide contents and the hydrogen-ion concentration of the polluted waters were measured. When an apple-snail was kept for 24 hours in a one-litre jar, about  $\frac{3}{4}$  full of water, the oxygen at the end of that period was 5.0 c.c. per litre, carbon dioxide 4 c.c. per litre and the pH about 7.8. As the larvæ depend mostly, if not entirely, on the atmospheric air for respiration, greater or lesser amounts of  $O_2$  and  $CO_2$  in the surrounding water should not affect them. The pH 7.8, as is well known, is also not harmful to mosquito larvæ. The pollution, therefore, appears to be due to the presence of toxic substances produced probably as by-products of putrefaction of the excreta of the snails. That the by-products of putrefaction do play an important rôle in polluted waters has already been shown by me elsewhere (1927). To discuss the nature of these by-products is beyond the scope of the present communication.

*Amphibia*—The tadpoles of the common frog *Rana tigrina* Daud seem to be very fond of eating mosquito larvæ. One full grown tadpole can consume about 15 to 21 larvæ in a period of 24 hours.

#### CONCLUSION

The above list of the enemies of the mosquito is far from being complete, inasmuch as only those forms have been tried which occur round

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# THE PENTAVALENT COMPOUNDS OF ANTIMONY IN THE TREATMENT OF KALA-AZAR

## Part III.

### AMINOSTIBUREA AN ANALYSIS OF THE TREATMENT IN 52 CASES

BY

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*The preparation*—The preparation is a combination of para-amino-phenyl stibinic acid with urea and glucose. It is supplied in ampoules in the form of a light brown powder, and is said to contain 24.8 per cent of metallic antimony. In the ampoules it undergoes no apparent change when kept at the Calcutta room temperature for a number of months during the hot weather. It is very hygroscopic and if the ampoule is cracked it rapidly absorbs water, becomes dark brown in colour and forms into a semi-fluid sticky mass. It is very easily soluble in water and in sterile distilled water it makes a sterile solution. No immediate change occurs in the solution provided it is kept sterile.

*Relative toxicity*—The relative toxicity of the compound is low. Using the English variety of white mouse of an average weight of 25 grammes, it was found that 7 out of 10 survived a dose of 125 milligrammes per kilogramme body-weight. With the Japanese variety of white mouse, average weight 10 grammes, the results of toxicity experiments were as follows—

Dose in grammes per kilogramme body-weight of mouse	Number of mice	Number surviving
100	5	5
150	5	5
200	5	5
250	5	3

Apart from the possibility that it may prove more efficacious than the cinchona alkaloids, it forms a very important starting point from which to conduct further researches into synthetic drugs

This drug, originally called Beprochin, has been placed on the market under the name of Plasmoquine or Plasmochin

The commercial product was originally the hydrochloride salt but as it was found that this was unsuitable for use in the tablet form, a salt with an acid of high molecular weight is now used. The acid portion is said to be therapeutically inactive. The tablets are issued in two forms (a) 'Plasmoquine' tablets, each of which contains the equivalent of 0.02 gm of the hydrochloride salt, and (b) 'Plasmoquine Compound' tablets, each of which is composed of 0.01 gm plasmoquine and 0.125 gm quinine sulphate

Very striking results have been claimed by many workers in the treatment of malaria with this drug (*vide* references), more especially in benign tertian malaria and for the destruction of crescents in the peripheral blood

The results recorded have chiefly dealt with the effect of the drug on the clinical symptoms of the disease and upon the rate of disappearance of parasites from the peripheral blood. No large series of cases seems to have been examined along the lines laid down by Sinton (1926a) for testing the efficacy of a drug in producing a permanent cure of malaria, although in many instances the patients have been observed clinically for some time after the end of treatment

Through the kindness of the makers, the I. G. Farbenindustrie Aktiengesellschaft of Leverkusen, Germany, we have been supplied with free samples of this drug and have been able to test it during the past year under the conditions laid down by Sinton (1926a). Our thanks are due to this Company

## DETAILS OF THE METHODS, ETC., USED IN THE EXPERIMENTS

The routine procedure used in testing the properties of plasmoquine were similar to those already described in detail in previous articles of this series (Sinton, 1926b)

The patients were all young adult male European soldiers from the same population mentioned in our other work and treated under similar conditions (Sinton, 1927)

## TREATMENT OF BENIGN TERTIAN MALARIA

All the patients were suffering from chronic benign tertian malaria and the disease was diagnosed by the finding of *P. vivax* in the peripheral blood immediately before the commencement of treatment. As in our other work a preliminary purgation with calomel and magnesium sulphate was given before the commencement of any course of treatment

### *Plasmoquine*

The original communication received from the makers recommended the following course of treatment — 'The daily dose of 0.02 gm plasmoquine, 3-4-5

died within 36 hours, a lumbar puncture showed that there was an acute pneumococcal infection of the meninges

The other was an advanced case of kala-azar, he died when he had been under treatment for about a fortnight

*Patients discharged as cured*—Of the patients discharged as cured we have been able to get information about 40, of these 2 died within a few months of discharge, possibly of some intercurrent disease as lung symptoms were prominent. The remaining 38 have received no further treatment and have remained in good health for 6 months or more after discharge

*Number of days under treatment*—The mean number of days under treatment of the 48 patients discharged as cured was 28.9, of the 40 previously untreated the mean number of days was 26, and of the 8 resistant cases 43.25

*Cessation of fever*—Of the 48 patients discharged as cured 2 were afebrile throughout the course of treatment, of the remaining 46 the number of injections given prior to the cessation of fever was 5.3. Of the previously untreated patients two were afebrile throughout, the mean of the number of injections given to the remaining 38 prior to the cessation of fever was 5.1, and to the 8 resistant cases was 6.4

*Splenic enlargement*—The average size of the spleen of the 48 patients before treatment was commenced was 4.2 inches below the costal margin, at the time of discharge the measurements were as follows—

Not palpable	11
Just palpable	28
2 in below costal margin	4
2½ in „ „ „	2
3 in „ „ „	2
4½ in „ „ „	1
	<hr/>
	48
	<hr/>

*Weight*—Two patients lost weight, 1 lb and 2 lbs, respectively, both of these were eventually cured. The remaining 46 patients gained an average of 7.0 lbs, the average gain amongst the previously untreated patients being 7.1 lbs and amongst the resistant patients 6.6 lbs

*The total leucocyte count*—In six instances this was not recorded. At the time of discharge in no instance was it less than 5,000 and in only 4 was it less than 6,000, the mean of the counts in 36 ordinary cases was 6,559 per c mm and in 6 resistant cases it was 8,146 per c mm. The mean in the whole 42 cases was 6,786

#### DOSAGE IN CASES IN WHICH A COMPLETE CURE WAS EFFECTED

Of the 38 patients who were completely cured 30 had previously received no treatment and 8 had previously received a full course of injections and had relapsed



Schulemann and Memmi (1927), from their study of the use of the drug on malarial patients, considered that the addition of quinine to plasmoquine was useful in preventing the cyanosis, sometimes seen during treatment. For these reasons larger daily doses of plasmoquine were used in these series of experiments.

*Series PMC 1*—The patients in this series were treated with daily amounts of plasmoquine compound equivalent to 0.10 gm plasmoquine and 1.25 gm quinine. The spacing of the doses was as in the PM 1 Series, so that the total amounts of plasmoquine given during the 17 treatment days was 1.70 gm per patient and of quinine 21.25 grms.

Fifteen patients were placed on this treatment and of these one was unable to complete the course on account of toxic symptoms.

*Series PMC 2*—The twenty patients in this series were given a 'continuous' course similar to that of the PM 2 Series. The daily dosage of plasmoquine was 0.10 gm and of quinine 1.25 gm, making a total of 2.8 grms plasmoquine and 35 grms quinine during the 28 days of treatment. The time needed to complete the course varied from 28 to 52 days with an average of 37.5 days per patient. All the patients completed treatment.

## RESULTS OF TREATMENT IN THE PREVENTION OF RELAPSE

Relapses were diagnosed as heretofore by the finding of parasites in the peripheral blood by the thick-film method of examination. These examinations were carried out weekly for at least eight weeks after the completion of all treatment (Sinton, 1926a).

*Plasmoquine Treatment*—Eight patients out of the twenty-nine in the PM 1 Series relapsed after treatment and two failed to complete the treatment on account of toxic symptoms. One case was lost sight of before the end of the period of observation after treatment. The observed relapse rate was 35.5 per cent with a possible maximum of 38 per cent and an observed minimum of 34.8 per cent (*vide* Sinton, 1926b, p. 583).

Three patients out of the twenty-two in the PM 2 Series relapsed and two others were unable to complete treatment because of toxæmia. The failure rate in this series was, therefore, 22.7 per cent.

*Plasmoquine Compound Treatment*—Out of the fifteen patients in the PMC 1 Series two relapsed and one could not complete treatment. The relapse rate was 20 per cent.

Eighteen of the twenty patients in the PMC 2 Series completed treatment without relapse, while the remaining two were lost sight of at the end of the 5th and 6th weeks of observation after treatment. The after-histories of these two patients were traced and it was found that they had shown no clinical signs of malaria up to the end of two months after we lost sight of them.

One patient who was admitted for malignant tertian malaria received 0.16 gm plasmoquine daily for 7 days, but relapsed with the same infection. He was then retreated with 0.10 gm plasmoquine and 1.25 gm quinine daily for 7 days, but on this occasion relapsed with benign tertian malaria in spite of the two courses of plasmoquine.

*The relative total dose*—As before the doses have been calculated according to the weight of the patient, the relative dose in this series being the dose per 100 lbs body-weight. The patients can be grouped according to the relative dose administered, as follows —

	Ordinary cases	Resistant cases
Less than 2 grammes	1	
2.0 to 2.49 grammes	7	1
2.5 to 2.99 grammes	2	1
3.0 to 3.49 grammes	9	
3.5 to 3.99 grammes	3	2
4.0 to 4.49 grammes	5	
4.5 to 4.99 grammes	2	1
Over 5 grammes	1	3
	30	8

The mean of the relative total dose given to the 38 patients was 3.58 grammes, to the previously untreated patients 3.30 grammes and to the resistant patients 4.64 grammes.

*Dosage according to age*—The relationship of the means of the actual and relative total doses to the age of the patients is shown below —

Age group	Number of patients	Mean actual dose, grammes	Mean relative dose, grammes
Under 10 years	8	1.56	4.18
10 years but under 20	13	2.55	3.48
20 years or over	17	3.04	3.41

There has been the same tendency in this series to give relatively larger doses in the case of children of the youngest age group.

*The cure-rate*—Although in no instance was it definitely ascertained that a relapse occurred amongst those patients who were discharged as cured, the two patients who died a few months after discharge must be looked upon as possible cases of relapse, these patients were given, respectively, 10 and 12 injections, actual doses of 2.4 and 2.7 grammes and relative total doses of 2.16 and 3.0 grammes. Neither of these patients had previously received any treatment. Of the two cases classed as failures, one had previously received no treatment, she was given 3.3 grammes in 14 injections, the relative total dose being 5 grammes. So that of the series of 33 patients who had previously received no treatment the cure-rate as far as it has been possible to ascertain was 90.9 per cent.

of 0.10–0.15 grm daily caused a quicker disappearance than did doses of 0.05 grm

In our experiments blood examinations were made by the thick-film method daily during the first week of treatment and once weekly thereafter until the end of observation

The records of the daily blood examination of 80 patients are available

Treatment	Total cases	Number of cases showing <i>P. vivax</i> after hours —					
		0	24	48	72	96	120
Plasmoquine	46	46	42	26	8	3	0
	Per cent	100	91	56	17	6.5	0
Plasmoquine compound	34	34	18	6	1	0	0
	Per cent	100	53	17	3	0	0

These figures are very similar to those of Fletcher (1927) in his plasmoquine series and show that plasmoquine has a destructive action on *P. vivax*. In no case were parasites found after the 4th day of treatment. The combination of the drug with quinine as plasmoquine compound has an even more rapid action, for only 3 per cent of the patients showed parasites on the 3rd day and none later. The average duration of parasites in the plasmoquine series was 1.71 days as compared with 0.73 in the compound series.

Some of our observations tend to confirm the findings of Roehl (1927) and of Muhlens and Fischer (1927) that gametocytes persist longer than schizonts.

### THE EFFECTS OF TREATMENT ON TEMPERATURE

Memmi and Schulemann (1927) state the fever in their cases disappeared in one to two days with a daily dose of 0.06 grm plasmoquine. Vad and Mohile (1927), using the same daily dosage, say that fever was generally controlled within 24 hours. Fletcher (1927) found with this dosage that none of his 46 patients had a temperature over 100°F after the 3rd day and all were normal on the 4th. Macphail (1927) says the temperature of his patients became normal not later than the 4th day.

The 'average duration of fever' (*vide* Sinton and Eate, 1926) amongst the 51 patients treated with plasmoquine only was 0.8 days. Thirty-eight or 74 per cent of them showed fever after the commencement of treatment, the 'average duration of fever' in the latter cases being 1.1 days. The maximum duration of fever in any case was 2½ days.

Thirty-five patients treated with plasmoquine compound had an 'average duration of fever' of 0.30 days. Fifteen or 43 per cent of these showed fever after the commencement of treatment, with an average duration of 0.70 days per case. One patient had fever for 3½ days, the long duration of which was probably due to the fact that he developed such severe diarrhoea and vomiting on the 2nd day of treatment that a temporary cessation was necessary.

When the results of the plasmoquine treatment are compared with those of the compound, it is seen that the febrifuge action of the latter is more marked. The results are very similar to those of the other workers quoted above.

In the 52 consecutive cases in which this drug was used the patient was discharged cured in 48 cases, of these, 38 were known to be in good health for at least 6 months after discharge, 8 were not traced and two died, possibly of some intercurrent disease

It is calculated, after making all allowances, that in a mixed population a total dosage of 2.3 grammes per patient or 3.31 per 100 lbs body-weight of patient should produce a cure-rate of not less than 90.9 per cent in that population

Although in the treatment of ordinary cases the drug compares well with the other pentavalent compounds of antimony which are available, its special value appears to lie in its action in resistant cases, in three instances a cure was effected in patients who previously had each had no less than three full courses of injections of different antimony compounds without being cured

My thanks are due to the Union Drug Co, Clive Street, Calcutta, for a generous supply of this compound for trial purposes

## THE EFFECTS OF TREATMENT ON THE GENERAL CONDITION OF THE PATIENTS

The weights of all patients were taken weekly while wearing the same clothing so as to avoid any marked error due to this factor

In the PM 1 Series, 61 per cent of the patients showed a gain in weight at the end of treatment. The average gain was 2.3 lbs per patient (max gain 10 lbs, max loss 4 lbs per case). In the PM 2 Series, 40 per cent gained weight but there was an average loss of a few ounces per patient (max gain 10 lbs, max loss  $4\frac{1}{2}$  lbs).

In the PMC 1 Series, 61 per cent had gained weight at the end of treatment. The average gain was 3.8 lbs per patient (max gain 10 lbs, max loss 4 lbs). In the PMC 2 Series, 70 per cent had gained weight at the end of treatment with an average of 1.5 lbs per case (max gain  $11\frac{1}{2}$  lbs, max loss  $7\frac{1}{4}$  lbs).

The percentage of all patients treated with plasmoquine alone who gained weight was 52, as compared with 66 in the compound series. The average gain in weight per patient in the two treatments was 1.2 lbs and 2.4 lbs respectively.

These figures seem to show that with the 'continuous' treatments although the 'cure rate' is higher, yet the improvement in general condition is not so rapid. The treatments with plasmoquine compound seem to have a better effect than those with plasmoquine alone.

Seventy-two per cent of 51 patients treated with quinine for 28 days gained weight. The average gain was 2 lbs per patient (max gain 11 lbs, max loss 8 lbs).

## THE EFFECT OF TREATMENT ON THE PREVENTION OF RELAPSE IN MALIGNANT TERTIAN MALARIA

Most observers have found that while plasmoquine produces very beneficial effects in benign tertian malaria, its action is much less marked in the malignant tertian type of fever.

*Plasmoquine Treatment*—One patient was treated with 0.08 gm plasmoquine daily according to the directions given by the makers (*vide* Series PM 1). The total amount of plasmoquine given during the 17 days treatment was 1.26 grms. This patient relapsed.

Three patients received a continuous daily dosage of 0.08 gm for 7 days and they all relapsed later. Three other patients were given the same dose daily but for 14 consecutive days and of these two relapsed. Another case received 0.16 gm daily for 7 days and also relapsed later.

The total number of cases treated with plasmoquine alone was 8 and of these 7 or 87.5 per cent relapsed after treatment.

*Plasmoquine Compound Treatment*—Two cases received 0.08 gm plasmoquine and 1.0 gm quinine daily for 7 consecutive days. Both of these relapsed. Two patients were given 0.10 gm plasmoquine and 1.25 gm quinine daily for 7 days and of these one had a relapse due to *P. falciparum* and one due to *P. vivax*. Another patient who received this dosage for  $8\frac{1}{2}$  days and one who received it for 14 days did not relapse.

# A SPLEEN-PUNCTURE SYRINGE

BY

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IN certain quarters spleen-puncture is looked upon as a dangerous operation. The present writer does not agree with this view, but admits that it is an operation which should not be undertaken lightly by the inexperienced. The success and safety of its performance are largely dependent on the dexterity of the operator.

It is not proposed to give a description of the technique of the operation, this will be found in a more suitable place (Napier, 1927). However, one of the first essentials is that the operator should know the exact position of the patient's spleen and be able to control accurately its movements during the operation. The usual practice is for the operator first to map out the position of the spleen and for an assistant to control the movements during the operation by placing his hand on the abdomen. This particular form of dual control has obvious disadvantages.

The syringe, or rather syringe attachment, which is described below, was introduced to overcome this difficulty. The syringe is held in one hand, and by a movement of the fingers the plunger can be withdrawn and released rapidly three or four times in the space of a second without altering the grip or moving the syringe (Plate XV, figs 1 and 2). If the plunger fits accurately and runs freely, as it always does in a properly prepared Roux syringe, the negative pressure in the barrel of the syringe causes it to spring back after each withdrawal. Meanwhile the other hand of the operator is entirely free to grip the spleen between the fingers and thumb, if it is a small movable one, or simply to control its downward movements if it is a large one.

This syringe has now been in use continuously for nearly seven years at the Calcutta School of Tropical Medicine, during this period the writer has used it in more than 2,000 spleen punctures. The majority of the patients have been

Manson-Bahr (1927) inside a week, Vad and Mohile (1927) in 6 to 7 days, Cordes (1927) in 1 to 6 days, average  $3\frac{1}{2}$  days

Fletcher (1927) found that the treatment usually tended to cause a diminution in the number of the parasites, but that they may disappear and reappear during treatment, and may be present as long as 12 days at least, in some cases Barmann and Smits (1927) and Polychromades (1927) had somewhat similar experiences and Muhlens (1927) also records such findings

These findings and the fact that such a high percentage of patients relapse after treatment would seem to indicate that it is not 'possible to banish the subtertian schizonts permanently from the blood with plasmoquine alone, and that even after large doses of the drug the schizonts reappear sooner or later'

(b) *Action on Sexual Parasites (Crescents)*—*Plasmoquine* Four crescent carriers were treated with doses of 0.08 gm plasmoquine daily. In one patient the gametocytes were absent at the end of 24 hours, while in the other three they persisted for 1, 3 and 5 days respectively after the commencement of treatment

*Plasmoquine Compound* Two patients were given 0.10 gm plasmoquine and 1.25 gm quinine daily and in one case crescents were absent at the end of 24 hours, while in the other they persisted for 48 hours. A third patient given 0.08 gm plasmoquine and 1.0 gm quinine daily showed these forms for 48 hours but not later

*Discussion of Results*—As noted previously, it is claimed that plasmoquine has a specific destructive action on crescents. The following are the times during which they have been recorded as persisting during treatment by different observers—Fischer and Weise (1927) about 10 days, Manson-Bahr (1927a, 1927b) 4 days, Shiwensky (1927) about 5 days, Schulemann and Memmi (1927) 6 to 8 days, Cherefeddin (1927) 5 to 6 days, Polychromades (1927) in 103 cases 2 to 8 days, Vad and Mohile (1927) 7 to 10 days, Radojcic (1927) 4 to 7 days, Manoloff-Sliven (1927) 2 to 3 days, and Pendlebury (1927) 3 to 7 days

On the other hand, Cordes (1927) found them present from 1 to 8 days and states that he could not demonstrate that plasmoquine prevented their formation during treatment. Barmann and Smits (1927) and Macphail (1927) record the appearance of these forms during treatment

The results recorded in our work, though few in number, help to confirm the claim that this drug has a destructive action on crescents. Previous work in this enquiry (Sinton, 1927c) went to show that the cinchona alkaloids have little destructive action on mature crescents, so although patients might be cured of their malignant tertian infections, yet they might still be potential carriers of infection to mosquitoes for a few weeks. The discovery of a drug which will rapidly destroy crescents, forms an important adjuvant to the treatment of malignant tertian malaria with these alkaloids

Plasmoquine, as noted above, appears to have little action on the asexual forms of *P. falciparum*, so patients treated with this drug only, are liable to parasitic relapses. We have found, as has already been recorded by Barmann and Smits (1927), that crescents reappear during the relapses, so the action of plasmoquine as a gametocide can only be a temporary one, while asexual parasites still persist in the body. We would, therefore, advocate in the treatment of malignant

Mr S Woodhouse, Officer-in-charge, Mathematical Instrument Office, Survey of India, Calcutta, has very kindly undertaken to make this syringe attachment for any Government institution at a moderate cost

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Sioli (1927) report a case of methæmoglobinæmia and in seven cases, where the daily dose of 0.10 gm. was exceeded, cyanosis was present accompanied in two instances by jaundice. This worker and Muhlens (1927) say that daily doses larger than this amount give rise to toxic manifestations. Manson-Bahr (1927a, 1927b) also notes that with doses greater than 0.08 gm. daily, cyanosis may occur which is undoubtedly toxic and due to methæmoglobinæmia.

Polychromiades (1927) with doses of 0.06 gm. daily, records cyanosis in 8 out of 46 patients. Muhlens and Fischer (1927) find cyanosis rare with daily doses of 0.04 gm., but say they occur with doses greater than 0.10 gm. daily. Schulemann and Memmi (1927) also record cyanosis, which they consider probably due to methæmoglobinæmia, and thought that it was a danger signal rather than actual evidence of poisoning. Several other workers have also recorded cyanosis.

Local epigastric tenderness and loss of appetite have been reported about the 4th or 5th day of treatment, but the makers state that 'these symptoms disappeared on withdrawing plasmoquine and did not as a rule recur when treatment was recommenced, so their presence did not constitute a definite contra-indication to the drug.'

Schulemann and Memmi (1927) state that abdominal pains may occur when large doses are given on an empty stomach, but seldom if a dose of 0.06 gm. daily is not exceeded. Polychromiades (1927) found that colic occurred about the 5th or 6th day of treatment and rarely after the 13th. Amongst 46 patients treated with plasmoquine he records colic in eight, while with plasmoquine compound 16 out of 142 developed this complaint between the 3rd and 6th days and five others between the 7th and 12th days, a total of 14.7 per cent. He says that 'trivial incidents like cyanosis and colic' were present in 23 per cent of his patients.

Manson-Bahr (1927) noted abdominal pains which he thought might be due to rapid contraction of the spleen. Manoloff-Sliven (1927) says that sometimes abdominal pains and diarrhoea occur and Vad and Mohile (1927) record pains in a few cases, but state they did not occur if the drug was given after meals.

Although cardiac symptoms are one of the signs of toxic action in animals, yet they seem to be rare or slight in the human subject. Fischer and Weise (1927) in their investigation of the toxicity of the drug found no such symptoms. Polychromiades (1927) found no change in blood pressure during treatment. Van den Braden and Henry (1927), however, record a case of tachycardia and Barmann and Smits (1927) found a slowing of the pulse after 5 to 7 days of treatment.

Albuminuria has been recorded, especially in those cases in which cyanosis was severe and methæmoglobinuria was present.

*Serious Complications and Deaths*—Cordes (1927) records a case of malignant tertian malaria with jaundice, which received 0.08 gm. plasmoquine and 1.0 gm. quinine daily. On the 5th day the jaundice increased, vomiting occurred and somnolence was a marked symptom. There was a drop of 35 per cent in the hæmoglobin of the blood. This patient died and on post-mortem a commencing

# SOME INSECT AND OTHER ENEMIES OF MOSQUITO LARVÆ

BY

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## INTRODUCTION

SINCE the end of the last century, when it was definitely established that the mosquito is undoubtedly the transmitting agent in the spread of malaria, numerous investigators, both in India and other countries, have tried to evolve methods for the control of this dangerous pest. The methods in vogue at present consist of suffocating and killing the mosquito larvæ by applying oil on the surface of water in which they are living, or by the use of poisons such as Paris green, etc. These methods, as is well recognised, besides giving only a temporary relief and that too only when they are faithfully and properly carried out, are fairly expensive. During the last decade or so, in the case of numerous insect pests, which had baffled entomologists for a long time, it has been shown that the surest and cheapest method of control is by means of the natural enemies of the pest concerned. In the case of the mosquito, however, with the exception of fish, very little attention has been paid to its natural enemies. I, therefore, very thankfully took up the suggestion of Lieut-Col R B Seymour Sewell, I M S, that I should conduct experiments in order to ascertain what aquatic animals, other than fish, would readily destroy mosquito larvæ.

### *Material and Methods*

The material for my experiments was collected from pools and tanks round about Calcutta. The Anopheline larvæ\* were supplied by Mr M O T Iyengar of the Public Health Department, Bengal.

The usual method of procedure was to introduce into a one-pound Kilner jar, containing fresh pond-water, a known number of larvæ, one or two specimens

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\* Mixture of *A sinensis* Wiedemann, *A barbirostris* van der Wulp, *A vagus* Dönitz, *A jamesi* Theobald, *A minimus* Theobald, *A fuliginosus* Giles, etc

sixteen or about 50 per cent were required by twelve patients during the first week. Parasites reappeared in the peripheral blood of eleven patients during treatment.

Seven of the fifteen patients in the PMC 1 Series required extra rests and one did not complete treatment. Among the remaining fourteen patients, the average number of days of rest needed was 10 days per patient. Eighty-five per cent of these rests were needed in the first week. The maximum duration of treatment in any one case was 32 days. In only one case did parasites reappear during treatment.

In the 'continuous treatments' twenty-one of the twenty-two patients in the PM 2 Series needed rests during treatment, and two could not complete the course. The number of days rest required by each patient varied from 0 to 25 with an average of 7.5 days per case. This means that an average of 35.5 days was needed to complete the 28 days 'continuous course'. Rests were not specially required during the first week as compared with the later weeks and in no case did parasites reappear during treatment.

In the PMC 2 Series, eighteen of the twenty patients needed rests but all completed the treatment. The number of extra days required varied from 0 to 24 with an average of 9.5 days per patient. As in the previous series, the days of rest were not more numerous during the first week as compared with the later ones, and no case showed parasites during treatment.

Toxic symptoms appeared most frequently about the fifth day of treatment and were usually only cyanosis and abdominal pains of varying intensity. These manifestations might appear separately or in combination. Abdominal pains were usually the first symptom to appear and then cyanosis, but cyanosis was often intense without any abdominal pains. A few patients showed intense pallor of the mucous membranes, especially those of the lips but this was not always accompanied or followed by cyanosis or abdominal pains.

The cyanosis, although taken to be a danger signal, did not seem to cause much discomfort to the patient. One case who had to take 25 days rest in order to complete his 'continuous treatment,' had intense cyanosis but no other symptoms. This patient was anxious to continue his treatment and complained on every occasion when it was stopped. Another became deeply cyanosed after 2 days of treatment and the condition did not disappear until he had rested for over a fortnight. As a general rule, however, toxic symptoms quickly disappeared when treatment was stopped.

No serious cardiac symptoms were seen but as noted by Barmann and Smits (1927), it was observed that the pulse rate after the first few days of treatment became slower than normal, usually about 65 per minute.

On those days when rests were needed on account of toxic symptoms the urine of all patients was examined as a routine measure. Albumen was detected in only one case, which was undergoing the 'continuous' PMC treatment. The albuminuria disappeared when a rest was given, but reappeared after treatment was recommenced.

Two patients in our series developed symptoms which gave rise to considerable anxiety. The first case was receiving a daily dose of 0.10 gram plasmoquine

larva and sucks out its blood by means of its long, sickle-shaped and hollow mandibles, which are pointed and perforated at the apices. A middle-aged larva of *Eretes sticticus* Linn destroys about 35 larvæ during one day. The dytiscid beetle itself attacks mosquito larvæ with its stout and toothed mandibles. One specimen of *Cybister limbatus* Fabr eats about 17 larvæ during 24 hours.

The *Hydrophilus* larvæ are still more voracious. The moment a mosquito larva was introduced into a jar containing a *Hydrophilus* larva, the latter at once seized it and completely ate it up, leaving not even its head capsule behind. A full grown larva of *Hydrophilus olivaceus* eats about 25 larvæ a day in this fashion.

Of the Gyrinidæ *Orectochilus gangeticus* Wied and a species of the genus *Dineutus* were tried and both proved to be deadly enemies of mosquito larvæ. On an average one specimen of *Orectochilus gangeticus* destroys 11 larvæ during one day. Considering the large numbers in which Gyrinid beetles occur in ponds, etc., there is no doubt that any Anopheles has but little chance of surviving in any area of water inhabited by these beetles.

*Odonata*—The larvæ of the following species very much relish mosquito larvæ —

<i>Anax guttatus</i> (Burm.)	fam. <i>Æschnidæ</i>
<i>Gynacantha</i> sp.	„
<i>Ictinus rapax</i> Ramb.	„
<i>Crocothemis servilia</i> Drury	<i>Libellulidæ</i>
<i>Bradynopyga geminata</i> Ramb.	„
<i>Ischnura senegalensis</i> Ramb.	<i>Agrionidæ</i>
<i>Pseudagrion microcephalum</i> Ramb.	„
<i>Ceragrion coromandelianum</i> Fabr.	„

It will be noticed that representatives of almost all the important families of this order destroy mosquito larvæ.

*Diptera*—Some of the most formidable natural enemies of mosquito larvæ are their own relatives. As is well known, the predaceous and cannibalistic larvæ of *Lutzia*, *Megarhinus*, etc., readily kill and eat them. For lack of material I have not been able to try any other dipterous form.

*Crustacea*—Several members of this group readily eat mosquito larvæ. When some larvæ are introduced into a jar containing the common freshwater shrimp, *Palæmon lamarrei* M-Edwards, the latter swims after one of them, takes hold of it by means of its mandibles, bends the body across and devours it completely. The larva, appearing as a U-shaped mass, can be seen through the transparent body lying in the stomach where it is gradually masticated. After it has been passed into the intestine the shrimp swims after and catches another larva. In this fashion a shrimp, about one inch in size, destroys about eleven larvæ in a day.

Crabs are also fond of eating mosquito larvæ, species like *Paratelphusa spinigera* Wood Mason and *Varuna litterata* M-Edwards being very useful in this respect. It, however, appears that their taste for mosquito food decreases with age, for whereas a young specimen ( $\frac{1}{2}$  in across carapace) of *Paratelphusa*

Fischer and Weise (1927) could only explain some of their results on the assumption that there was a personal idiosyncrasy to the drug in some instances. The experience gained in our work tends to confirm the idea that some patients are more susceptible to the effects of the drug than are others. Some patients were able to complete a continuous course of treatment lasting 28 days without a rest, while others needed very numerous rests. The patient who received 0.16 grm daily did not develop any toxic symptoms until the 7th day of treatment, while many cases on half this daily dose exhibited them by the 4th day or in some instances as early as the 2nd day. There seems to be little doubt that there is a personal idiosyncrasy to plasmoquine in some cases.

Some observers consider that the occurrence of cyanosis and abdominal pains are of little importance, while others think them to be danger signals rather than actual evidence of toxæmia. In consideration of the findings of different workers and our own experiences, we think that these symptoms are undoubtedly due to toxic effects and should be taken as a warning to diminish the dosage of plasmoquine or, to stop, temporarily at least, the administration of the drug. Although the addition of quinine to plasmoquine seems to have some effect in diminishing the toxicity of the drug, it does not appear to have the very marked action which some work would lead one to believe. Larger doses and 'continuous treatment' give rise to more toxic effects than do smaller doses and the 'interrupted treatment'.

The safety margin of the drug seems to be small, more especially when one takes into account the question of personal idiosyncrasy, and doses of 0.10 grm and, perhaps, even doses of 0.08 grm daily are, in our opinion, too large for *general* use. In this connection it is interesting to note that the makers have since recommended that the dosage should be reduced to 0.06 grm daily (Hennings, 1927). Before the drug can be taken into general use, much more work is necessary under carefully controlled conditions to determine the best dosage and the duration of administration.

Up to the present the results of the treatment of less than 1,000 patients with this drug have been recorded, yet at least three deaths are known to have occurred as the result of the action of plasmoquine and several cases of severe toxic effects have also been reported. If such events have occurred under the strict and careful conditions in which the drug has been tested, it seems to us that the time has not yet arrived when it can be given broadcast for use in general practice, however useful it may be under hospital conditions or under very strict and *daily* medical supervision. In the present state of knowledge of the action of this drug it is essential, in our opinion, that every patient under this treatment should be seen, at least, once daily so that administration can be stopped as soon as the first signs of toxæmia are detected.

## SUMMARY

The results of our investigations may be summarised as follows —

(1) After the treatment of 51 patients suffering from chronic benign tertian malaria with plasmoquine only, 30 per cent relapsed, while amongst 35

about Calcutta. Even so, the above account reveals that many animals hitherto unsuspected are powerful destroyers of mosquito larvæ. The way in which molluscs injuriously affect the larvæ is quite novel and it will be interesting to see if any other animals behave in the same fashion. My chief object in publishing the present communication is to draw the attention of mosquito workers to the value of the biological method of control of this serious pest.

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# STUDIES IN MALARIA, WITH SPECIAL REFERENCE TO TREATMENT

## Part IX.

### PLASMOQUINE IN THE TREATMENT OF MALARIA

BY

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AND

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(*Quinine and Malaria Enquiry, Malaria Survey of India, Kasauli* )

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MACGILCHRIST (1914) states that quinine is a combination of quinoline with a piperidine ring, and Horlein (1926) says it is held to consist of a quinoline nucleus to which is attached a second ring system, the so-called chinucludin residue. The latter worker looks upon quinine as a complicated alkyl-amino-6-methoxy-quinoline.

Many attempts have been made in the past to produce quinine by synthetic means and within the last few years the German chemists, Drs Schulemann, Schonhofer and Wingler, have manufactured a synthetic derivative of quinoline, which they state to be a salt of alkyl-amino-6-methoxyquinoline, with an analogous formula to quinine. Roehl (1926) tested the value of this drug in bird malaria and found it about 60 times as active as quinine. It has since been tried by numerous workers in the treatment of human malaria with apparently very beneficial results in some forms of this disease.

The discovery of a synthetic drug, which has a marked destructive action on the malaria parasite, is a most important event and very careful tests are necessary to determine to what extent it can be used to replace the cinchona alkaloids which up to the present have proved the most useful drugs in the treatment of malaria.





times, should not be given more than 5 days in succession. The after-treatment is carried out in the same manner as that of quinine, viz ,

For 5 days plasmoquine

4	„	rest
3	„	plasmoquine
4	„	rest
3	„	plasmoquine
4	„	rest
2	„	plasmoquine
5	„	rest
2	„	plasmoquine
5	„	rest
2	„	plasmoquine
5	„	rest

*'Precautionary measures*—When convulsive pains of the stomach or cyanosis of the lips are noticed after the administration of Plasmoquine, it should immediately be stopped and only given again when symptoms have completely disappeared'

The recommended treatment, therefore, consisted of 17 days of treatment and 22 days rest, a total of 39 days, provided more rests were not required on account of toxic manifestations

*Series PM 1*—The dosage recommended for strong adults was 0.06 to 0.10 gm daily. In our cases the doses were graded as follows—Patients weighing 7 stone received 0.05 gm daily, those of 8 stone 0.06 gm, those of 9 stone 0.07 gm, those of 10 stone 0.08 gm, those of 11 stone 0.09 gm and those of 12 stone or over 0.10 gm. The majority of our patients weighed about 10 stone each, so the usual daily dose was about 0.08 gm daily on the days when treatment was given or an average total dosage of 1.26 grms per patient. The spacing of the doses was as recommended above.

Twenty-nine patients were placed on this treatment and in two of them it was discontinued on account of persistent toxic symptoms.

*Series PM 2*—It was suggested in one of the circulars issued by the makers that better results might be obtained if the drug was administered continuously, as far as toxic symptoms would permit. It was, therefore, decided to try a series of patients on a 'continuous' treatment, to last until the drug had been given on 28 days with as few rests as possible.

Twenty-two patients were started on this treatment, in which the daily dosage was 0.08 gm plasmoquine irrespective of the weight of the patient. The total amount of plasmoquine received by each patient was 2.24 grms and the time needed to complete the 28-day course varied from 28 to 53 days, with an average of 36 days. In two cases treatment had to be discontinued.

#### *Plasmoquine Compound*

Eichholtz (1927), as a result of animal experiments, considered that quinine tended to lessen the toxic effects of Plasmoquine. Manson-Bahr (1927b) and

erect ones which may be easily overlooked. These erect hairs are present in both sexes but are more abundant in females. In rubbed specimens the scanty erect hairs may have disappeared but the distinctive scars left can easily be seen in specimens stained and mounted in balsam (Sinton, 1927a)

*Appearances in Stained and Mounted Specimens*

The measurements of the Type Female and of three others are given in Table I. The remarks, etc., in this table show the variations in the ratios, etc., which were found amongst the eleven specimens measured.

TABLE I  
*Phlebotomus clydei* n sp. Type ♀

Structure		Length in mm. of specimens number				Remarks, relative lengths, etc †
		1*	2	3	4	
BODY	Clypeus and head	0.457	0.400	0.471	0.414	
	Thorax	0.643	0.643	0.700	0.557	
	Abdomen	1.400	1.314	1.457	1.271	
	Total length	2.50	2.36	2.63	2.23	
	Labium	0.228	0.228	0.243	0.228	= 2.75 — 2.9 × breadth
	Epipharynx	0.214	0.221	0.228	0.214	
	Pharynx, length	0.153	0.174	0.180	0.162	
	Pharynx breadth	0.066	0.063	0.064	0.063	
ANTENNA	Segment III	0.141	0.138	0.150	0.138	< IV + V
	Segment IV	0.075	0.075	0.081	0.078	IV + V + VI < XII to XVI
	Segment V	0.075	0.075	0.078	0.078	XII to XVI > 2 × IIIrd
	Segment VI	0.075	0.075	0.078	0.078	Formula $\frac{2}{III-XV}$
	Segments XII-XVI	0.336	0.321	0.327	0.315	
	Total length	1.214	1.214	1.257	1.173	= 8.5 — 8.7 × IIIrd, = 3.7 — 3.8 × XII — XVI, = 0.68 — 0.72 × wing, = 0.41 — 0.44 × leg

\* Type female

† Data from 11 specimens

*Quinine Treatment (Control)* —The observed relapse rate of 111 patients treated with quinine was 77 per cent. During the last five years over 1,000 patients suffering from benign tertian malaria have been treated in this enquiry by means of the various cinchona alkaloids with a relapse rate of about 70 per cent.

### *Discussion of the Results of Treatment*

Among the fifty-one patients treated with plasmoquine alone, the relapse rate averaged about 30 per cent. It was found that as the duration of treatment was increased from 17 to 28 days, the relapse rate fell from 36 per cent to 23 per cent. Muhlens (1926) reports a relapse rate of 31 per cent after plasmoquine as compared with 59 per cent after quinine.

Amongst the thirty-five patients treated with plasmoquine compound, the relapse rate was only 8.5 per cent. The rate amongst the patients who received the shorter course was 20 per cent, as compared with no recorded relapses among those who received the longer course.

The relapse rate amongst the eighty-six patients treated with plasmoquine alone or in combination with quinine was only about 21 per cent, which is a remarkably low rate when compared with that amongst the controls and with those of other workers on treatments with quinine (Stephens, etc., 1917—1919, Acton, etc., 1921).

The combination of quinine with plasmochin seems to be a distinct advantage and the results of the last treatment with plasmochin compound are so remarkable that they will require confirmation on a larger series of patients.

There seems, however, to be no doubt that in plasmochin a distinct advance has been made in the treatment of chronic benign tertian malaria, but, as will be seen from the section on toxic symptoms, the margin of safety in the dosage of the drug seems to be small.

### THE EFFECTS OF TREATMENT ON THE DURATION OF *P. VIVAX* IN THE PERIPHERAL BLOOD

Many workers have confirmed the statement of the makers that treatment with plasmoquine causes a rapid disappearance of *P. vivax* from the peripheral blood. Some of the previous records may be summarised thus —Roehl (1926) parasites disappeared in 72 hours, Schulemann and Memmi (1927) on 3rd to 4th days, rarely persisting till 6th, Barmann and Smits (1927) in 3 to 80 hours, average 36 hours, Pendlebury (1927) in 31 cases, in only one lasted after 3rd day and none after 4th, Manson-Bahr (1927) in 24 hours, Vad and Mohile (1927) in 4 to 7 days, and Muhlens and Fischer (1927) in 3 to 5 days, Shwensky (1927a) in 2 to 7 days and Djokic and Stambuk (1927) in 1 to 5 days.

Fletcher (1927) gives an interesting table detailing the results of a dose of 0.06 grm plasmochin daily to 46 cases. Of these 87 per cent showed parasites at the end of 24 hours, 39 per cent after 48 hours, 19.6 per cent after 72 hours, and 2 per cent after 96 hours but none later.

Roehl (1926) and Muhlens and Fischer (1927) found that the gametocytes persisted slightly longer than the schizonts and the latter authors record that doses

The *buccal cavity* (Plate XVI, fig 4) shows a well-marked triangular pigmented area and the armature consists of 15 large sharply pointed teeth. The *pharyngeal armature* (Plate XVI, fig 5) consists of a series of parallel transverse ridges. Anteriorly these are unarmed but further back these are seen to carry short spines, which more posteriorly become long and needle-like. The arrangement is very similar to that seen in *P. hospiti* and *P. christophersi*. The length of the pharynx is about 2.75 to 2.90 times its breadth.

The *palps* (Plate XVI, fig 1) have a formula of 1, 2, 4, 3, 5 and the relative lengths of the segments are 3, 7.9, 12.6, 10, 22.3. The 4th segment is approximately equal to the combined lengths of segments 1 and 2. The 3rd segment, which is slightly incrassate, is shorter than the IIIrd antennal segment. The 'curious spines' of Newstead number about 30 and are situated on the distal portion of the basal third of the 3rd segment. The ratio palp over epipharynx is 3.0—3.2.

The *antennae* (Plate XVI, figs 2 and 3) have a total length equal to 8.5—8.7 times that of the IIIrd segment. The formula is 2 over III to XV. The geniculate spines are comparatively short but reach almost to the succeeding inter-segmental articulation. The length of III is less than half the combined lengths of XII to XVI.

The *wing* (Plate XVI, fig 6) is about 4 times as long as broad. The length  $\beta$  is about equal to  $\gamma$  and in five specimens  $\beta$  was equal to  $\epsilon$ . The ratio  $\alpha$  over  $\beta$  is 0.6—0.8 and  $\delta$  over  $\alpha$  0.2—0.4.

The length of the *hind leg* is about four times that of the femur and three times that of the tibia.

The *genitalia*—The spermathecae (Plate XVI, fig 7) are crenulated and closely resemble those of the other members of this division of the erect-haired group (Cf *P. hospiti* and *P. christophersi*, Sinton, 1927a). The post-genital ridge (Plate XVI, fig 8) carries 4 spines.

### Differential Diagnosis

The crenulated spermathecae and the erect dorsal abdominal hairs distinguish this species from all the members of the recumbent haired group (Sinton, 1927a).

The scanty erect hairs on the abdomen, the well-developed buccal armature and pigmented area, as well as the morphology of the spermathecae differentiate *P. clydei* ♀ from *P. papatasi*, *P. major*, *P. argentipes*, *P. newsteadii* and *P. sergenti*.

The presence of scanty erect hairs and the shape of the spermathecae are similar to *P. hospiti* and *P. christophersi* but the morphology of the buccal armature and the pigmented area are distinctive (Cf Sinton, 1927b). In *P. hospiti* the teeth are smaller and more numerous and the pigmented area is not so markedly triangular. In *P. christophersi* these structures are poorly developed.

### *Phlebotomus clydei* n. sp. (♂)

In Table II are given the measurements of the Type and three other males. The remarks, etc., in this table show the variations among ten specimens measured.

We have records of the treatment of 1,127 British cases treated for chronic benign tertian malaria with different cinchona alkaloids during the past 5 years. The 'average duration of fever' amongst these cases was 0.31 days. These results tend to show that plasmoquine alone has not so marked an action in reducing fever in malaria as has the cinchona alkaloids. However, when plasmoquine is combined with quinine in doses of 15 to 20 grains daily, the febrifuge effects seem equal to those of the cinchona alkaloids in benign tertian malaria.

### THE EFFECTS OF TREATMENT ON SPLENIC ENLARGEMENT

Schulemann and Memmi (1927), Manson-Bahr (1927) and Brosius (1927) all lay emphasis on a very rapid decrease in the degree of splenic enlargement in patients treated with this drug and Macphail (1927) records a similar reduction in children. Manson-Bahr (1927) thinks that the epigastric pain complained of by some patients may be due to the rapid decrease in size of this organ. Barmann and Smuts (1927), Shwensky (1927a) and Polychromades (1927) also report beneficial effects on enlarged spleens.

The degree of splenic enlargement in our cases was determined weekly. The examinations were made in the recumbent position and the results recorded in 'finger-breadths' beyond the costal margin. The size of the 'average spleen' and of the 'average enlarged spleen' were calculated by giving a value of 0 to non-palpable spleens, a value of 1 to spleens which were palpable but not enlarged beyond the costal margin, a value of 2 to spleens extending one finger-breadth beyond the costal margin and so on.

The splenic index before treatment commenced was 43 per cent among the 49 patients treated with plasmoquine only and was about 16 per cent at the end of treatment. Whilst among the 34 patients treated with the compound the indices were 50 and 20.6 per cent respectively.

The 'average spleen' in the plasmoquine series was 1.18 before and 0.28 after treatment, as compared with 1.35 and 0.32 in the compound series. The 'average enlarged spleen' in the former series was 2.76 before treatment and 1.75 afterwards, while in the latter series it was 2.71 and 1.28 respectively. The differences in the degree of reduction in the two series is not marked, but is rather in favour of the compound.

On account of the high cure rate produced by the drug one would not be surprised to get the very rapid reduction in size of the enlarged spleen reported by other workers. It has not, however, been our experience that the reduction in size of the enlarged spleen in benign tertian malaria is more marked or more rapid than after treatment with the cinchona alkaloids. Amongst 1,187 patients treated by the latter form of treatment, the splenic index was 48 per cent before and 7 per cent after treatment. The 'average spleen' was 1.25 before and 0.124 afterwards and the 'average enlarged spleen' 2.62 and 1.76 respectively. These figures do not support the suggestion that plasmoquine treatment has any more marked action on splenic enlargement than have the older methods of treatment.

TABLE II—(contd)

Structure	Length in mm of specimens number				Remarks, relative lengths, etc †	
	1*	2	3	4		
Wing.	Length	1 540	1 685	1 640	1 770	= 4 1—4 2 × breadth, = 0 60—0 62 × leg
	Breadth	0 371	0 414	0 400	0 428	$\frac{\alpha}{\beta} = 0 48-0 74, \frac{\beta}{\gamma} = 0 9-1 2$
	α	0 185	0 214	0 171	0 243	$\frac{\alpha}{\beta} = 0 55-0 75, \frac{\delta}{\alpha} = 0 14-0 37$
	β	0 293	0 314	0 357	0 321	$\frac{\alpha}{\gamma} = 0 60-0 68, \frac{\beta}{\epsilon} = 0 83-1 25$
	γ	0 314	0 300	0 285	0 321	$\frac{\theta}{\epsilon} = 2 3-2 8 \frac{\alpha + \beta}{\theta} = 0 61-0 68$
	δ	0 043	0 043	0 028	0 071	Wing length $\frac{\theta}{\epsilon} = 2 02-2 20$
	ε	0 285	0 350	0 285	0 357	
	θ	0 714	0 785	0 814	0 814	
	π	0 043	0 114	0 114	0 100	
HIND LEG	Femur	0 657	0 685	0 657	0 728	= $\frac{1}{4}$ length leg
	Tibia	0 900	0 928	0 943	0 985	= $\frac{1}{3}$ length leg
	Tarsus, segment 1	0 485	0 500	0 485	0 500	
	Tarsus, segments 2 5	0 600	0 657	0 614	0 643	
	Total length	2 64	2 77	2 70	2 86	(Not including coxa and trochanter)
GENITALIA	Sup clasper, seg 1	0 258	0 271	0 271	0 271	= 2 3—2 4 × seg 2, = 1 0—1 05 × inf clasper
	Sup clasper, seg 2	0 111	0 117	0 114	0 114	
	Intermed append	0 210	0 216	0 225	0 225	= 0 78—0 88 × inf clasper
	Intromitt organ	0 072	0 063	0 066	0 060	
	Inferior clasper	0 243	0 264	0 255	0 270	
	Sub gen lamellæ	0 174	0 189	0 189	0 195	= 0 70—0 76 × inf clasper
	Genital filament	0 180	0 039	0 114	0 240	(Distance protruded)

\* Type male

† Data from 10 specimens.

The total number of cases treated with plasmoquine compound was 6 of which 3 relapsed as malignant tertian malaria and one as benign tertian

*Quinine Treatment*—Seven control cases were treated with the quinine and alkali treatment (Sinton, 1926b) for one week and of these only one relapsed

*Discussion of Results*—A total of 14 cases suffering from malignant tertian malaria have been treated with plasmoquine alone or in combination with quinine and of these 10 or 71 per cent have had relapses due to *P falciparum*. Amongst 7 control cases treated with quinine and alkali the rate was only 14 per cent. Shwensky (1927) reports 50 per cent of relapses after plasmoquine treatment (22 patients) and 30.4 per cent after the compound (125 patients).

The three cases which did not relapse in the two plasmoquine series were those which received a 'continuous' treatment lasting more than 7 days. The results in the plasmoquine compound series were better than those in the plasmoquine series but the number of cases is too few to generalise upon, and in neither case do they compare favourably with the quinine and alkali treatment (Sinton, 1926b).

#### THE EFFECTS OF TREATMENT ON THE DURATION OF *P. FALCIPARUM* IN THE PERIPHERAL BLOOD

The makers in one of their circulars state that 'Plasmoquine acts specifically on the gametocytes (crescents) of the subtertian parasite, but it was not found possible to banish the subtertian schizonts *permanently* from the blood by plasmoquine alone. Even after large doses of the drug the schizonts reappeared sooner or later in the blood'. This seems to be the experience of most workers who have tried the drug and it is for this reason that the combination of quinine with plasmoquine is advocated in malignant tertian malaria.

(a) *Action on Asexual Parasites—Plasmoquin*. The duration of these forms in the peripheral blood of 6 patients treated with 0.08 gm plasmoquine daily was as follows—In two cases parasites were present until the end of the 7 days of treatment, in one case undergoing 14 days treatment the parasites disappeared on the 3rd day but reappeared on the 12th, in one case they were present for 48 hours and in another for 24 hours, while in the last case they had disappeared at the end of 24 hours. In the case which received 0.16 gm daily they disappeared inside 24 hours.

*Plasmoquine Compound*. Two cases treated with 0.08 gm plasmoquine and 1.0 gm quinine daily showed parasites for less than 24 and 48 hours respectively. Three cases receiving 0.10 gm plasmoquine and 1.25 gm quinine showed parasites for 12, 24 and 48 hours respectively.

*Discussion*—Muhlens (1926), Muhlens and Fischer (1927), Schulemann and Memm (1927) and van den Braden and Henry (1927) found that plasmoquine had little effect on the sexual forms of *P falciparum*. The disappearance of these forms following this treatment has been recorded, but how much of this is due to the natural defences of the body and how much to an action of the drug is doubtful. These findings may be summarised as follows—Muhlens and Fischer (1927) say they may disappear in 7 days, Fischer and Weise (1927) in 4 to 6 days,



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tertian malaria one week with quinine and alkali (*vide* Sinton, 1926b) accompanied or followed by one week of treatment with plasmoquine

### TOXIC MANIFESTATIONS

Most workers who have reported on plasmoquine treatment have recorded the occurrence of toxic manifestations. The usual ones are an ashy-grey cyanosis of the lips, gums and finger nails and abdominal pains. In several instances, however, more severe manifestations have been seen in the form of an exacerbation of the symptoms just mentioned, accompanied by vomiting, jaundice, albuminuria and collapse. This severe toxæmia has in some cases been followed by death.

#### *Records by Previous Observers*

*Mulder Manifestations*—Muh lens (1926) and Memmi and Schulemann (1927) have suggested that the symptoms were not due to an intoxication with the drug, but to vaso-motor disturbances in nervous patients. Unfortunately, in the light of more recent work this theory cannot be accepted.

Eichholtz (1927) and le Heux and van Wyngaarden (1927) have tested the toxicity of this drug on animals and found that in toxic doses it gives rise to cardiac irregularity, slow and irregular action of the heart, marked cyanosis, dyspnoea and in some cases convulsions. The recovery was usually rapid, if a fatal result did not ensue. The effects varied in different animals and in cats the cyanosis was accompanied by methæmoglobinæmia.

Sioli (1927) and Manson-Bahr (1927) report that, in those of their patients who had marked cyanosis, methæmoglobinæmia was also present.

Fischer and Weise (1927) made an extensive investigation into the cause of the cyanosis during plasmoquine treatment. They found that methæmoglobinæmia could always be detected in the blood of 25 out of 26 patients a few days after the commencement of treatment, while treatment was continued and for some time afterwards. They could discover no relationship between the dosage of plasmoquine and the amount of methæmoglobin in the blood, but a marked one between the dosage and the time of appearance and duration of this condition. As a rule the intensity and duration increased with prolonged administration. The intensity with the same dosage usually varied inversely with the number of red blood cells, so that the greater the degree of anæmia the greater the amount of methæmoglobinæmia.

The condition remained within certain limits with the ordinary doses and they think it is of no importance. They were unable to find any sign of severe general disturbance related to any particular organ. Some of their findings showed such striking variations that they could only explain these as personal idiosyncrasies.

These workers concluded that it was the alkyl-amino group which was responsible for the causation of the methæmoglobinæmia, as in the case of acetanilid and phenacetin poisonings, but that with plasmoquine one did not get the unpleasant symptoms found with these drugs.

#### EXPLANATION OF PLATE XVI

Camera lucida drawings of the parts of *P. clydei* ♀.

- Fig 1 Palp showing Newstead's spines
- „ 2 Segments XIII to XVI of the antenna
- „ 3 Segments II to IV of the antenna
- „ 4 Posterior portion of the buccal cavity showing the teeth (T) and the  
pigmented area (P)
- „ 5 Pharynx
- „ 6 Wing
- „ 7 Spermatheca
- „ 8 Post-genital plate

necrosis of the liver cells was found. Similar symptoms, but in a milder degree, were encountered in three other patients.

Barmann and Smits (1927) gave a patient 0.08 gm. plasmoquine daily and on the 3rd day he developed cyanosis with a temperature of 102.5°F and became unconscious. The urine contained albumen and the patient died next day. On post-mortem some necrosis of the liver was found.

Vad and Mohile (1927) report that one of their cases died of pneumonia on the 5th day of treatment and Colonel S. P. James in the discussion at the 7th Congress of the Far Eastern Association of Tropical Medicine mentioned that he knew of another death following plasmoquine treatment.

Eiselberg (1927) records that he saw a patient who had received a total of 0.2 gm. plasmoquine during three days and then complained of much epigastric pain. After taking the last pill he vomited and lost consciousness. On the next day he was still vomiting, his liver was tender, his urine contained much albumen and there was a great fall in the number of red blood cells. This case recovered.

A patient treated by Sioh (1927) with 0.06 gm. daily complained on the 8th day of hepatic pain and weakness. He was cyanosed, had methæmoglobinæmia and collapsed when he attempted to leave bed. With a daily dosage of 0.06 gm. plasmoquine, two of the cases recorded by Fletcher (1927) became alarmingly ill with a rise of temperature, cyanosis, vomiting and collapse.

Manson-Bahr (1927*a*, 1927*b*) records three cases with toxic symptoms. One case had received a total of 0.40 gm. of plasmoquine in daily doses of 0.12 gm., when he developed cyanosis, with clammy sweats and abdominal pains. There was methæmoglobinuria and albuminuria. During the attack there was a great destruction of red blood cells which was followed by jaundice and 'resembled a mild blackwater-fever which ran a favourable course'. The second case had pains in the abdomen and back, nausea and cyanosis after 0.08 gm. daily for 3 days. The third case had slight cyanosis, but marked abdominal pain after a dosage similar to that in the first case.

#### *Records in Present Experiments*

It is evident from the literature just reviewed that the administration of plasmoquine is liable to be followed by toxic symptoms of a greater or lesser severity even with doses of 0.10 gm. or less daily. In our patients the occurrence of distinct cyanosis or of abdominal pains was considered to be an indication for a rest in the course of treatment and the number of rests affords an indication of the extent of the toxic symptoms encountered by us. Some cases did not complete treatment, either because of the severity of the toxic symptoms, or because so many rests were found necessary that parasites were not eliminated from the peripheral blood.

Of the twenty-nine patients in the PH 1 Series, twenty-two needed extra rests in addition to those on the schedule of treatment. Four of these did not complete treatment. Excluding these four patients the total number of extra days of rest needed was 33 or an average of 1.3 days per patient. The maximum duration of treatment in any one case was 32 days. Of the 33 days rest required,



and 1.25 gm quinine, when on the morning of the third day of treatment he developed an ashy-grey pallor with slight cyanosis, vomiting and severe abdominal pains. He had 12 motions during the day and his temperature rose to  $103^{\circ}\text{F}$ . On the next day his temperature was  $102.2^{\circ}\text{F}$  in the morning and fell to normal in the evening. The severity of the abdominal pains was less, but he passed over 20 motions during the day and was collapsed. On the following day he had improved and quickly recovered.

The second case, who was on the 'continuous' PM treatment and receiving 0.08 gm daily, developed even more alarming symptoms. On the 25th day of treatment he was brought to hospital with vomiting, collapse, severe diarrhoea and subnormal temperature, in fact symptoms closely resembling an attack of cholera. This patient had been quite well when given his last dose earlier in the day and apparently his attack had come on without any warning. He had previously stood the treatment very well and had only required two single days of rest previously. His condition caused considerable anxiety for some days, but he eventually recovered and did not subsequently relapse with malaria.

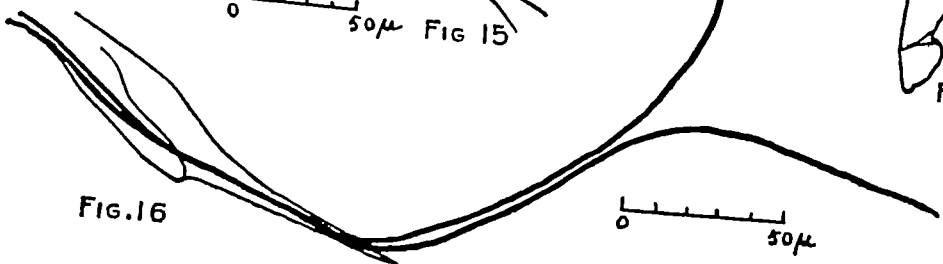
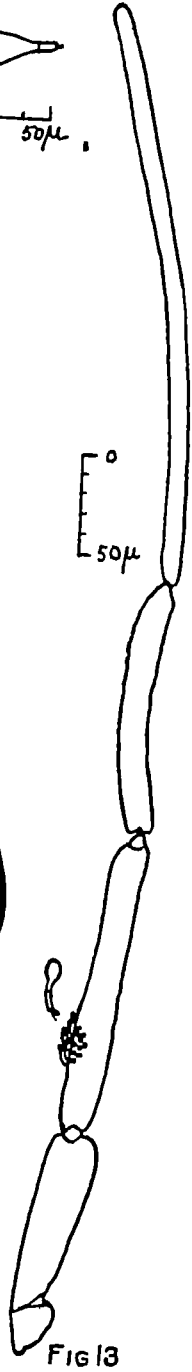
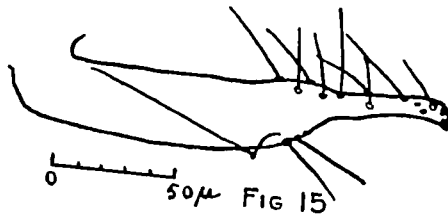
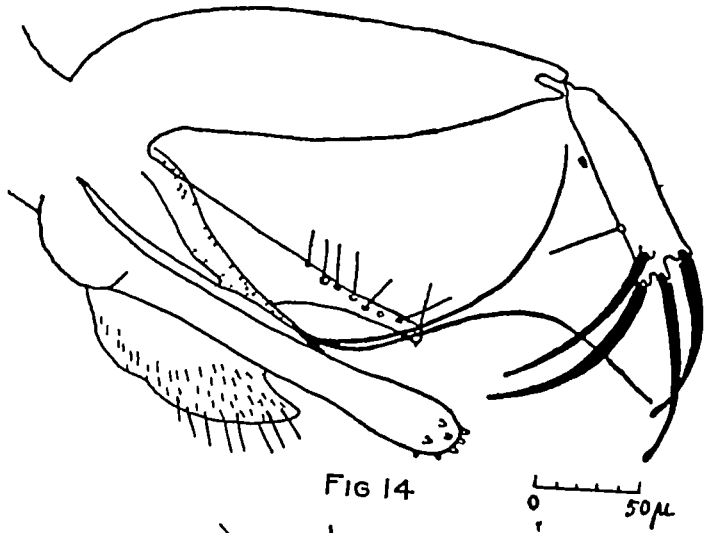
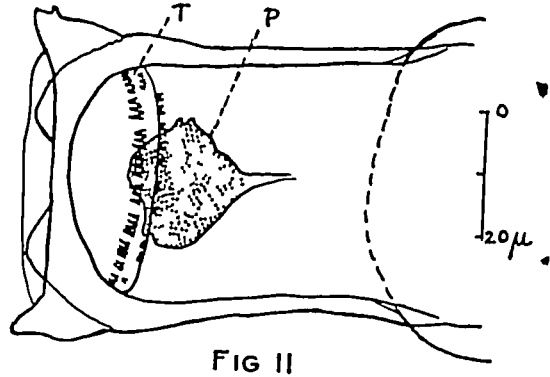
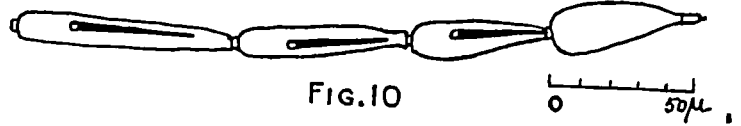
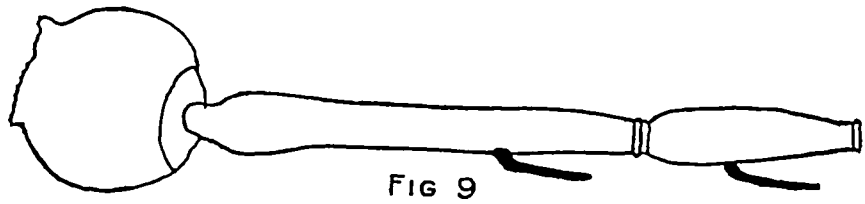
The very marked action of sugar and alkali in protecting patients against the toxic effects of stovarsol (Sinton, 1927), suggested that these substances might also protect against the deleterious effects of plasmoquine. To test this possibility, alternate patients were treated with and without these substances, but the results of the two series of 36 patients each showed no very decided differences.

### *Discussion of Toxæmia*

It was seen, under the conditions of our experiments, that in the 'interrupted series' toxic symptoms were more common during the first week than in the subsequent ones, but that no such difference could be established in the 'continuous treatment'. The statements of some workers would seem to imply that after the first week of treatment a certain degree of tolerance to the drug was acquired, but this has not been our experience. One would expect in the 'interrupted courses' given by other workers that fewer toxic symptoms would be observed after the first week, because rests were given and the duration of each course of treatment was decreased, while the length of the rests was increased. The chances of the drug accumulating in the body would under these conditions be diminished after the first week. In the 'continuous treatments' there would be no such diminution in the chances and this would account for the symptoms of toxæmia being more evenly scattered over the whole course of treatment. It seems to us, therefore, that there is no evidence to show that any marked degree of tolerance to the drug is acquired and that the supposed tolerance is simply due to a diminution in the amount of the drug given and an increase in the number and duration of the rests.

Le Heux and van Wyngaarden (1927) found that some animals developed toxic symptoms much more readily than others when given equal doses per kilogram of body-weight and thought that in the former case this was due to a much less rapid destruction of the drug inside the body, resulting in an accumulation and thus toxic symptoms. Manson-Bahr (1927*b*) found that toxic symptoms might occur after comparatively small doses in susceptible patients and

# PLATE XVII



patients treated with plasmoquine compound the rate was 8.5 per cent, as compared with about 70 per cent amongst control cases treated with quinine.

(2) 'Continuous treatment' seems to have a greater effect in producing a permanent cure in benign tertian malaria than has the 'interrupted treatment,' although more liable to be followed by toxic symptoms.

(3) A 71 per cent relapse rate was observed in 14 cases of malignant tertian malaria treated with either plasmoquine alone or in combination with quinine, while amongst 7 control cases treated with quinine and alkali the rate was only 14 per cent.

(4) Plasmoquine rapidly removes all forms of *P. vivax* from the peripheral blood and also the sexual forms of *P. falciparum*, but seems to have little action on the asexual forms of the latter parasite.

(5) Plasmoquine did not have as rapid effect in the reduction of temperature as did quinine, but when given in combination with the latter drug its febrifuge action is enhanced.

(6) Severe toxic symptoms may follow the use of plasmoquine and the margin of safety with the present dosage seems to be comparatively small.

### CONCLUSIONS

(1) The discovery of plasmoquine has marked a distinct advance in the treatment of malaria, but, as pointed out by Manson-Bahr (1927a), this drug should be 'regarded as the beginning, not the climax, of a new series of anti-malarial drugs.'

(2) In our experiments plasmoquine was much more effective in producing a permanent cure in benign tertian malaria and in abolishing crescents from the peripheral blood than quinine, but in the treatment of attacks of malignant tertian malaria the latter drug is still the treatment of choice.

(3) The low margin of safety in the dosage of plasmoquine renders it necessary that further experiments should be carried out to determine the best dosage and duration of treatment, before the drug is issued for general use outside hospitals.

Our thanks are due to the Director of Medical Services in India for the facilities which he has placed at our disposal for carrying out this work and to the Indian Research Fund Association who provided the money to cover the expenses of this investigation.

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The observations were made in the district areas of the province of Bihar and Orissa

I am greatly indebted to the Government of Bihar and Orissa, for placing every facility in the way of my investigation, and to the Maharajah of Tekari and his officers, for liberal help in conducting the investigation among the agricultural tenants in the Gaya district area

My thanks are due to Captain P J Barraud of the Central Research Institute for helping me in the mosquito work

Finally, I am thankful to my own staff consisting of Captain K K Das, Civil Sub-Assistant Surgeon Babu Kulamoni Misra, and Stenotypist Mr Gopal Krishna Chopra, for their assistance, zeal and loyal support

## II MATERIAL AND TECHNIQUE

### *Material*

The investigation has been tabulated under the following scheme The population of Bihar and Orissa has been classified into industrial, institutional and agricultural The agricultural group has further been divided according to the physiographical conditions of an area, situated at or above the sea-level This group of population might be either sparse or dense in any area The sparse conditions were mainly due to mountains, forests, marshes and unculturable areas The density was due to the regions being cultivated Cultivation depended upon the water-supply from the rains, canals and river inundations

The district areas of Bihar and Orissa were grouped according to the above scheme and the agricultural group of the population investigated accordingly

The work, especially in the agricultural group of the population, which always is beset with difficulty, was carried out by first selecting a type area, secondly, by visiting the police posts which contained the type area, and, thirdly, by examining the population collected at the police post on a particular date

The co-operation of the Local Medical Officer who is usually stationed in charge of the District Board Hospital in such places was also enlisted

The examination was conducted usually during the night between the hours of 8 p m and 1 a m Fresh preparations of the peripheral blood were examined and the diagnosis of the presence of microfilaria was made on the spot The cases in which microfilariae were found were classed as 'positive,' the rest as 'negative'

The cases selected for examination were males The leading signs and symptoms were of the following nature

- |       |                  |   |
|-------|------------------|---|
| (i)   | A long duration  |   |
| (ii)  | History of fever |   |
| (iii) | Enlargements     | <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">Glandular</div> <div style="display: inline-block; vertical-align: middle;">Genital</div> <div style="display: inline-block; vertical-align: middle;">Terminal</div> </div> <div style="display: inline-block; vertical-align: middle; font-size: 2em;">}</div> <div style="display: inline-block; vertical-align: middle;">Upper and lower extremities.</div> </div> |
| (iv)  | Effusions        | <div style="display: inline-block; vertical-align: middle;">Nature</div> <div style="display: inline-block; vertical-align: middle; font-size: 2em;">{</div> <div style="display: inline-block; vertical-align: middle;"> <div>Permanent.</div> <div>Fugitive</div> </div>  |

# NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

## Part XXIII.

### *PHLEBOTOMUS CLYDEI* n sp

BY

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(*Malaria Survey of India*)

[Received for publication, March 29, 1928]

THROUGH the kindness of Captain D Clyde, I M S, a large number of specimens of *Phlebotomus* were received from Waziristan. Amongst these were some insects belonging to the division of the 'erect-haired' group of *Phlebotomus* in which the erect hairs on the dorsum of the abdomen are scanty in number. This species has been carefully compared with the other known Indian members of this division—*P hospitu* and *P christophersi*—and been found to be a distinct species. It is proposed to call the species *Phlebotomus clydei*.

#### *Material*

The following specimens of this species have been examined —

(a) Six females and seven males collected at Jandola, Waziristan, on 18th August, 1923 (Type male and female included)

(b) One female collected in the Officers' Rest Room, Fort, Jandola, on 27th July, 1923

(c) Three females and three males collected in the Rest Camp, Khirgi, Waziristan, on 16th August, 1923

(d) In addition to these one female was found amongst the specimens collected by me in Waziristan in 1920 (Sinton, 1922) but was not differentiated at the time

#### *Phlebotomus clydei* n sp (♀)

The insect is yellowish brown in colour and of medium size. The majority of the hairs on the dorsum of the abdomen are recumbent but there are some

4 On the top of the lamp glass, place also some dates, split open, or slices of banana, or raisins, both this fruit and strip of folded lint should rest on

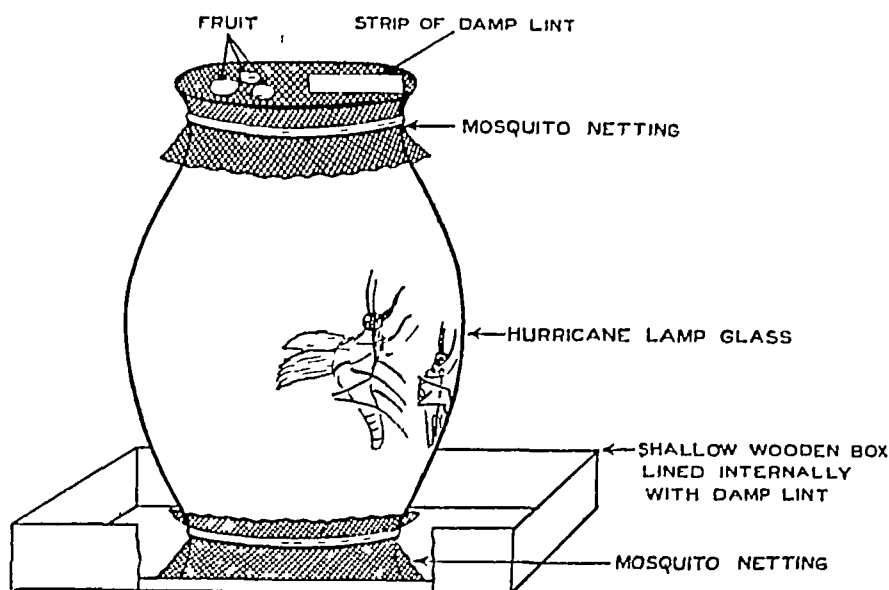


Fig 1 —Receptacle for keeping mosquitoes alive at the laboratory

the mosquito netting covering the top of the lamp glass, but not so as to completely cover it as this would prevent access of air

Mosquitoes kept in the above manner should survive for a considerable period, but may require to be kept cool in the very hot weather

The fruit and mosquito netting should be renewed if they become mouldy

### III RESULTS OBTAINED IN FIELD STUDIES

#### (a) FILARIAL INCIDENCE IN THE ARIAS OF BIHAR AND ORISSA

Out of 1,254 cases examined, which belonged mainly to the agricultural section of the population, 150 were found to contain microfilariae in the peripheral blood, i.e., about 12 per cent

Out of 203 cases, showing definite signs and symptoms of filariasis, 50 were positive for microfilariae or about 25 per cent

Out of 1,051 cases not showing any signs and symptoms of filariasis, 100 were positive for microfilariae or about 10 per cent. The result indicates that in an endemic area one person out of ten acts as a 'hidden' danger to the community in the spread of the filarial disease and this point needs no further emphasis

The following table (Table 1) shows the filarial incidence in the areas investigated. It also shows the leading clinical signs and symptoms which were present in the cases

Further, the table is subdivided into A and B, A gives detailed information about a group of cases residing in an area and in whose blood microfilariae were detected, B gives detailed information about a group of cases residing in an area

TABLE I—(contd.)

Structure	Length in mm of specimens number				Remarks, relative lengths, etc †
	1*	2	3	4	
Segment 1	0 036	0 030	0 036	0 036	Formula 1, 2, 4, 3, 5
Segment 2	0 096	0 102	0 099	0 093	Relative lengths 3, 7 9, 12 6, 10, 22 3
Segment 3	0 156	0 162	0 159	0 150	< IIIrd, > 1 + 2
Segment 4	0 123	0 129	0 123		= 1+2
Segment 5	0 270	0 285	0 300		$\frac{P}{E} = 3 0-3 2$
Total length	0 681	0 717	0 717		
Length	1 757	1 700	1 857	1 630	= 3 95-4 10 × breadth, = 0 60 - 0 62 × leg
Breadth	0 424	0 414	0 457	0 400	$\frac{a}{\beta} = 0 61-0 80, \frac{\beta}{\gamma} = 1$
α	0 200	0 200	0 257	0 243	$\frac{a}{\gamma} = 0 61-0 91, \frac{\delta}{a} = 0 2-0 4$
β	0 328	0 328	0 328	0 285	
γ	0 328	0 328	0 328	0 278	$\frac{a}{\epsilon} = 0 60-0 65, \frac{\theta}{\epsilon} = 2 1-2 5$
δ	0 035	0 057	0 085	0 071	$\frac{\beta}{\epsilon} = 0 64-1 0, \frac{a+\beta}{\theta} = 0 64-0 67$
ε	0 328	0 328	0 414	0 380	
θ	0 828	0 800	0 871	0 814	$\frac{\text{Wing length}}{\theta} = 2 0-2 14$
π	0 057	0 071	0 071	0 071	
Femur	0 714	0 685	0 757	0 685	= $\frac{1}{4}$ length leg
Tibia	0 914		1 057	0 857	= $\frac{1}{4}$ length leg
Tarsus, segment 1	0 500		0 528		
Tarsus, segments 2-5	0 643		0 671		
Total length	2 77		3 00		(Not including coxa and trochanter)

\* Type female

† Data from 11 specimens

The *total length* of the insect varied between 2 30 and 2 63 mm. There are about 10-14 erect hairs on the 3rd abdominal segment and the number decreases on the succeeding segments.

In Table I-A, 80 per cent of cases and in Table I-B, 85 per cent of cases gave a history of duration which was found to be long and extending over years

The combination of *signs* and *symptoms* most commonly met with, were the history of fever and the effusion in the tunica vaginalis

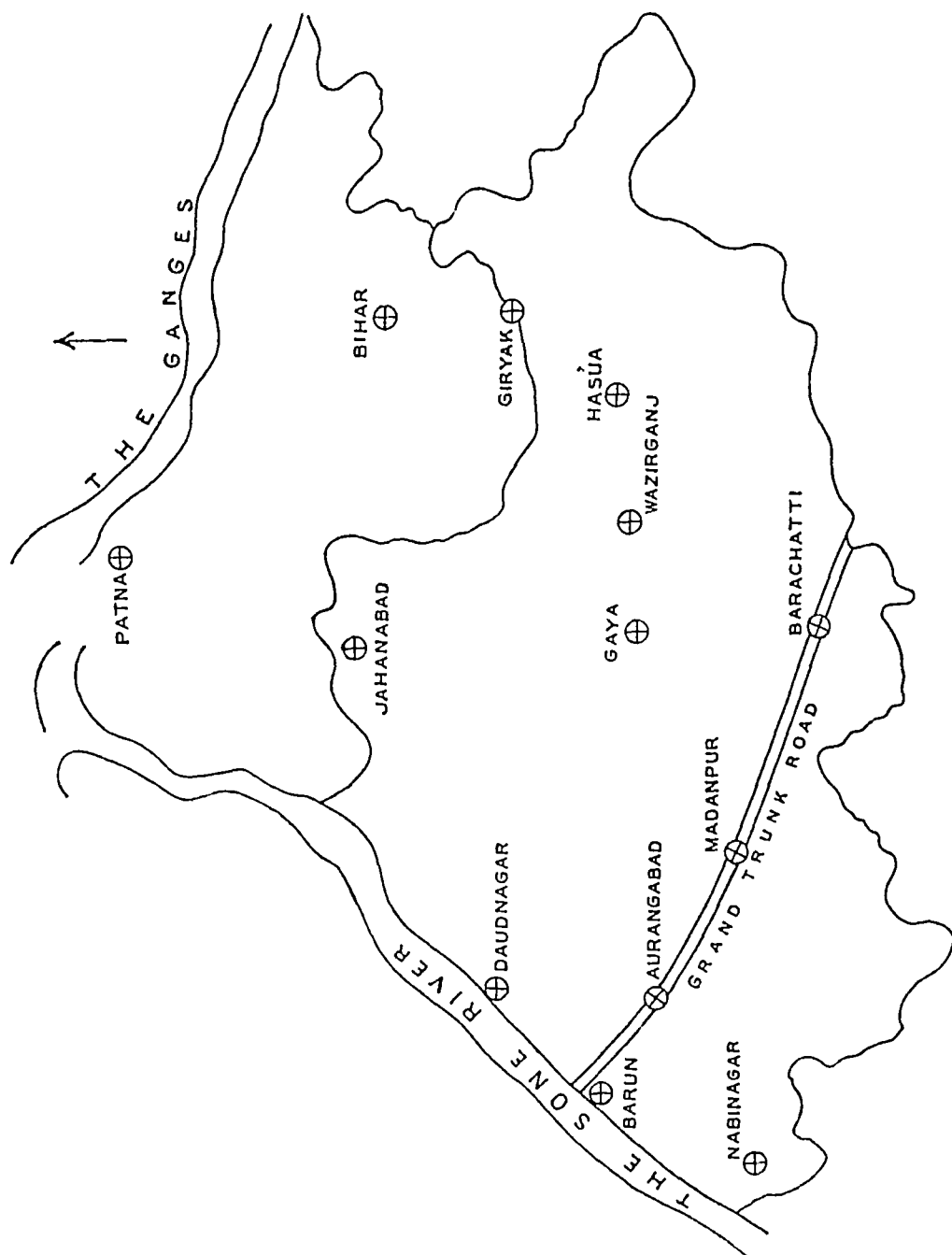


Fig 2.—Filariasis, Area of Bihar and Orissa, Gaya and Patna Districts, 0—5 miles

Next came the clinical features relating to the enlargements of the lower extremities and scrotum

The enlargements of inguinal glands, upper extremities, testes and fugitive œdemas were represented in their order of frequency

The *total length* of the insect varied from 2 10 to 2 90 mm The erect dorsal abdominal hairs numbered about 4 to 6 on the 3rd segment and fewer on the more distal ones

TABLE II  
*Phlebotomus clydei* n sp Type ♂

Structure		Length in mm of specimens number				Remarks, relative lengths, etc †
		1*	2	3	4	
BODY	<i>Clypeus and head</i>	0 385	0 443	0 328	0 443	
	Thorax	0 571	0 628	0 571	0 643	
	Abdomen proper	1 171	1 430	1 371	1 543	
	Sup clasper, seg 1	0 258	0 271	0 271	0 271	
	Total length	2 4	2 77	2 5	2 9	
Labium		0 200	0 228	0 230	0 228	
Epipharynx		0 185	0 190	0 193	0 200	
ANTENNA	Segment III	0 153	0 165	0 162		< IV + V
	Segment IV	0 081	0 090	0 093		XII to XVI = 2 × IIIrd
	Segment V	0 084	0 093	0 093		<u>1</u>
	Segment VI	0 084	0 090			Formula III–XV
	Segments XII XVI	0 318	0 333			
	Total length	1 243	1 285			{ = 7 8–8 1 × IIIrd, = 3 0 × XII – XVI, = 0 76–0 80 × wing, = 0 47 × hind leg
PALP	Segment 1	0 030	0 030	0 036	0 036	Formula 1, 2, 4, 3, 5
	Segment 2	0 090	0 090	0 096	0 099	Relative lengths 2 7, 7 5, 12 7 10, 22 7
	Segment 3	0 153	0 159	0 159	0 168	= IIIrd, > 1 + 2
	Segment 4	0 120	0 123	0 120	0 132	= 1 + 2
	Segment 5	0 288	0 270	0 270	0 312	$\frac{P}{E} = 3 5-3 7$
	Total length	0 681	0 672	0 687	0 747	

\* Type male

† Data from 10 specimens





The *buccal cavity* (Plate XVII, fig 11) has a pigmented area which is less developed than the same structure in the female. The buccal armature consists of a row of small teeth with another interrupted one of poorly developed teeth anterior to it. The pharyngeal armature resembles that in the female but is smaller.

The *palps* (Plate XVII, fig 13) have a formula of 1, 2, 4, 3, 5. The relative lengths of the segments are 2.7, 7.5, 12.7, 10, 22.7. The 3rd segment is approximately equal to the IIIrd antennal. Newstead's spines number about 30 and are situated slightly more distal than these structures in the female. The ratio palp over epipharynx is 3.5—3.7.

The *antennæ* (Plate XVII, figs 9 and 10) show a formula of 1 over III to XV. The length of the IIIrd segment is approximately equal to half the combined lengths of XII to XVI. The total length of the antenna is relatively greater than in the female.

The *wing* (Plate XVII, fig 12) is about 4.1—4.2 times as long as broad. As in the female  $\beta$  is equal to  $\gamma$  and in half the specimens  $\beta$  was almost equal to  $\epsilon$ . The other ratios are shown in the table.

The *hind leg* is about 4 times as long as its femur and 3 times its tibia.

The *genitalia* (Plate XVII, figs 14 to 16) are of the *minutus* type. The proximal segment of the superior clasper about equals the length of the inferior clasper and is about 2.3—2.4 times that of the distal segment. The sub-genital lamellæ are not quite  $\frac{3}{4}$  the length of the inferior clasper. The intromittent organ has a sharply pointed end. The genital filaments are markedly protruded and have pointed ends.

#### Differential Diagnosis

The morphology of the superior clasper and its spines distinguish this species from all the members of the erect-haired group except *P hospitu* and *P christophersi*. It can be distinguished from both these by the morphology of the buccal cavity, and also from the former by its palpal formula 1, 2, 4, 3, 5 not 1, 2, 3, 4, 5.

The absence of scales on the pleuræ, the morphology of the buccal cavity and pharyngeal armature, the antennal and palpal formula and the absence of Newstead's spines from the 2nd palpal segment differentiate it from *P squamipennis*.

The erect hairs on the dorsum of the abdomen distinguish *P clydei* from the members of the *minutus* group, the genitalia of which have a somewhat similar morphology. In addition the morphology of the buccal cavity and pharyngeal armature are different from that seen in *P montanus*, *P minutus*, *P babu*,\* and *P shortu*, the Indian species in the *minutus* group.

\* A re-examination of the type of *P babu* Annandale, 1910, from the Indian Museum, Calcutta, has shown that it is not identical with the European species, *P minutus* Rond, as was believed by Annandale and therefore must again be raised to specific rank as *P babu* Annandale. This species with its variety, *P babu* var *niger*, is the one which has commonly been identified in India in the past as *P minutus* and *P minutus* var. The type *P minutus* Rond in India seems to be confined to the western areas of the country, while *P minutus* var *antennatus* is much more widely spread.

Case Police Constable (under the care of Lieut-Colonel P S Mills, I M S, Civil Surgeon, Gaya), age, 42, caste, Kayastha, clinical signs, attacks of fever, presence of hydrocele, and anæmia, embryos of *bancrofti* found in numbers in the peripheral blood

The case was admitted to the Police Hospital, Gaya, and a two-hourly examination of a measured quantity of blood (20 cmm hæmoglobinometer pipette) was conducted and the total parasites were counted. The following is the count

Date	Hour	Number of parasites
2nd February 1928	13	1
	15	7
	17	3
	19	9
	21	6
	23	56
3rd February 1928	1	54
	3	65
	5	62
	7	20
	9	4
	11	0

During the phase of 24 hours, the parasites practically disappeared from the peripheral blood for nearly three hours between 9 and 12. They began to appear in progressive numbers for twelve hours between midday and midnight and reached the maximum figure for six hours between 24 and 6.

It is the last period which in all probability is the most dangerous hour for contracting the infection and propagating the disease.

In the line of treatment and in prevention, one has to take a note of this 'danger zone' in Filarial Periodicity.

(g) The relation between the total number of granules and the length of the embryo in microns

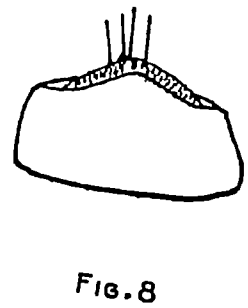
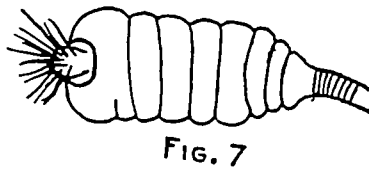
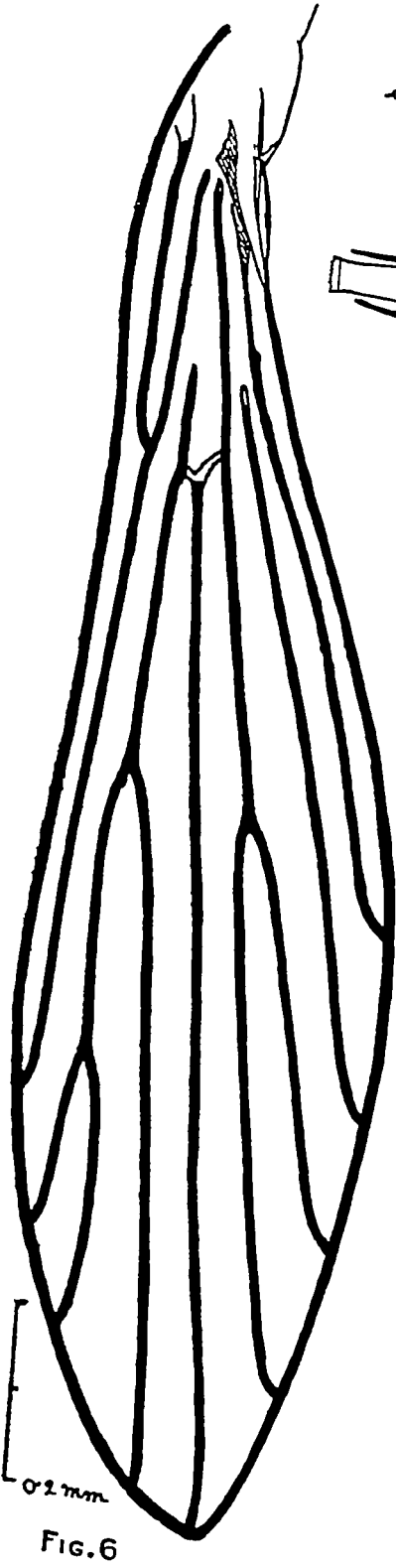
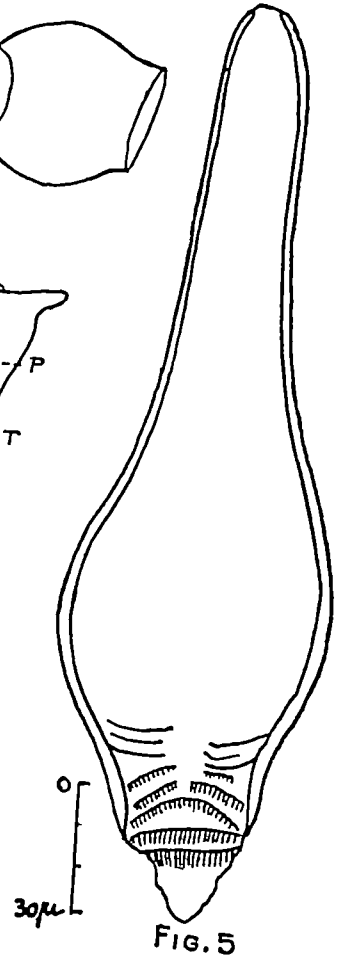
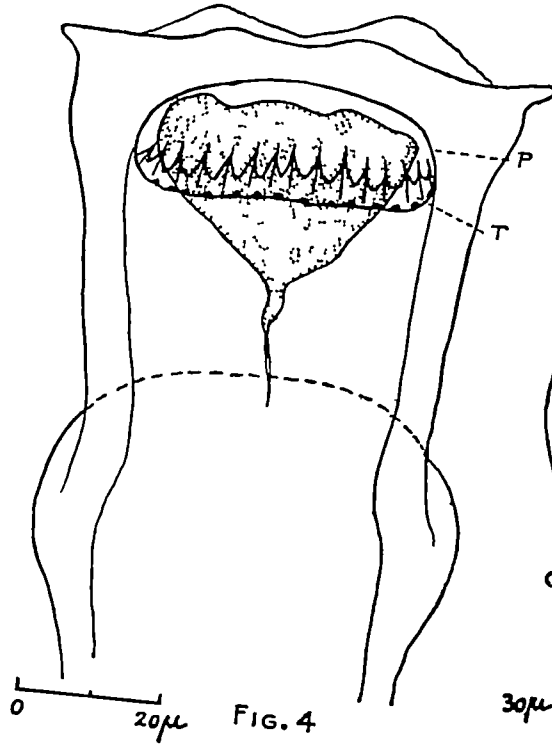
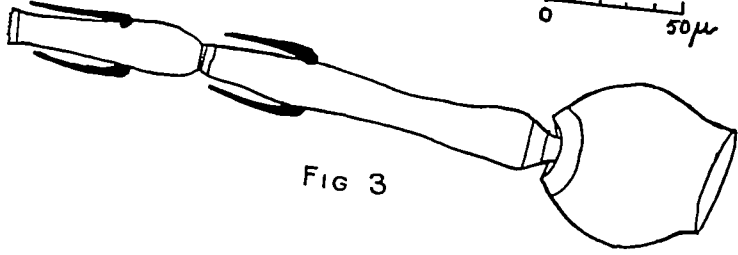
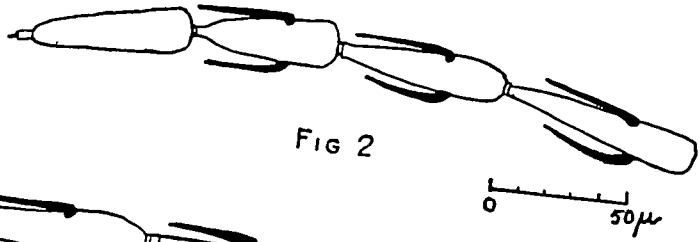
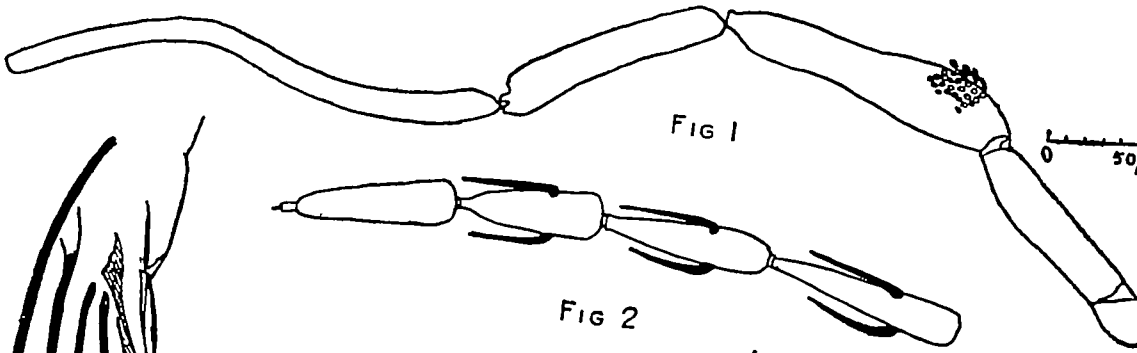
I do not know whether attention has been drawn to the above point. The granules in the body of *Microfilaria bancrofti* are fine, distinct and separate. They are therefore easily countable in each section of the anatomical points.

There are two wide sections in the cylindrical body of the parasite, viz, the cephalic space and the space between L, S, Z and S which are free from granules. In addition there are 3 minor gaps, viz, the nerve ring, the excretory pore and the anal pore, which are sometimes wholly, more often partially, free from granules. The rest of the sections are filled with granules. In many cases there appears to be some relation between the total number of granules and the length of embryo in microns, the former exceeding the latter by about 10 in the majority of cases. This relationship is found both in the human and mosquito phases of the embryo but is more pronounced in the former. Thus it has been found in 33 out of 50 counts in the human phase and 5 out of 12





# PLATE XVI



It was not possible to say with certainty when the parasite was ingested by the mosquito, it might have been minutes, it might have been hours, but the character of the human blood indicated that the blood was fresh and had not undergone disintegration. Mosquitoes belonging to the same batch, caught at the same time and in the same place, showed the embryos with and without sheaths. It is reasonable to presume therefore, that the first change in the life-cycle, i.e., *ecdysis* had commenced soon after the ingestion of the human blood by the mosquito.

The results of observation in the mosquito are tabulated under Table IV.

As seen from the table the developmental changes of *bancrofti* can be classified broadly under three forms, viz., (a) the form found in the human phase, (b) the form showing a cylindrical and sausage-shaped body with the remnant of a tail and (c) a long slender filari-shaped body.

The first form was observed in the alimentary tract and the last two in the thoracic muscles of the mosquito.

The movements of the first two forms were sluggish, the movements of the last form were vigorous, attended by a change of place.

(a) First form, like the human phase but devoid of sheath (*vide* Experiments 3, 4, 6 to 9, 12, 16, 23 and 24).

In some specimens the granules were coarse, large and countable. They were arranged along the cuticular wall so as to suggest the formation of an intestinal canal (Experiment 6, fig 5). In others the forms were thicker and stouter and the large, thick and coarse granules filled the body cavity (Fig 3).

Some forms were shrivelled and suggested the appearance of degeneration (Figs 2 and 4). These forms were observed after a few hours, and after the first, second and fifth days of captivity.

(b) Second form, the second form appeared as (i) cylindrical sausage-shaped bodies in which the alimentary canal was visible but the mouth parts and A. P. were not visible (Experiment 16, figs 9 and 10), (ii) forms like the above, but where the alimentary tract and mouth parts were both visible (Experiment 25, figs 12, 14 and 16), (iii) forms similar, but with the body a cellular mass, with no definite structures visible (Experiments 11, 12, 18 and 19, figs 11 and 13).

These forms were observed after a few hours, and after the 1st, 2nd, 3rd, 4th, 5th, 7th, 9th, 12th and 14th day of captivity.

(c) The third form was filari-shaped, about 1 mm in length and showed vigorous movements. It was seen after the 3rd day of captivity (Fig 17).

#### IV DISCUSSION OF RESULTS

In order to arrive at a true situation of the filarial condition of an area one has to consider the sequence of events which goes to determine the economic aspect of the agricultural section of the population of the area.

The economic aspect is the result of physical features, temperature, moisture, rainfall and the character of the chief cultivation of an area.





Age, Develop- mental stages	Results in Mosquito		Morphological Notes	Reference to Figures in Plate XVIII
	Negative number	Positive number		
1 day	10		Negative	Figs 4, 7, 9, 10, 13 and 14
3 days	15		Negative	
2 days		2	2nd day—Human phase, 314 $\eta$	
5 "		1	5th day—Human phase, 265 $\times$ 9 $\eta$ , also bodies vary- ing 116—134 $\times$ 9—15, tail remnant	
7 "		1	7th to 9th day—The rudimentary alimentary canal deeply stained, mouth parts and cesophagus not distinguishable, cellular mass deeply stained, anal area free from staining, remnant of tail	
9 "		2		
2 "		1		
4 "		1	14th day—Bodies 275—420 $\times$ 25—40 M showing canalization alimentary tract	
	1		Negative	
	4			
	4			
3 days	4		4th day—Bodies 150 $\times$ 15 M cellular, <i>vide</i> Experiment	
	9	2	16	
5 "	5			
Few hours	1		Bodies 170 $\times$ 25 M <i>vide</i> Experiment 16	
1 day	7	2		
Few hours	3		Negative	Figs 11 and 17
Few hours	11	1	<i>Vide</i> description in the explanation of figures	
3 days	11	1		
Few hours	1		Negative	Figs 8, 12, 15 and 16
1 day	18			
3 days	19			
Few hours	8	4	Few hours—Microfilariae human phase, also bodies	Fig 3
3 days	20	9	650 $\times$ 50 M formation of intestine mouth parts not distinct, showing remnant tail	
5 "	1	1	3 to 5 days—Bodies varying 750—1,075 $\times$ 35—30 microns	
Few hours	1		2 days—Microfilaria human phase	Figs 6, 18 and 19
2 days	5	1		
Few hours	20	4	Bodies showing alimentary canal, cesophagial bulb, mouth parts, lining cells of intestine not visible, 300—425—700 $\times$ 45—25—35 M	
Few hours	4		Bodies in the thoracic muscle as in Fig 6	
1 day	7	1		

#### EXPLANATION OF PLATE XVII

Camera lucida drawings of the parts of *P. clydei* ♂.

- Fig 9 Segments II to IV of the antenna  
„ 10 Segments XIII to XVI of the antenna  
„ 11 Posterior portion of the buccal cavity showing the teeth (T) and  
pigmented area (P)  
„ 12 Wing  
„ 13 Palp  
„ 14 Genitalia  
„ 15 Intermediate appendage  
„ 16 Intromittent organ and genital filament

had developed into stages possessing a definite alimentary canal and were situated in the thoracic organ of the intermediary host

- 11 Fifteen per cent of *Culex fatigans* showed developmental stages of *Microfilaria bancrofti* in the Gaya district area in the months of February and March
- 12 Twelve per cent of mosquitoes, species not determined, showed developmental stages of *Microfilaria bancrofti* in the Puri town

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*Ind Jour Med Res*, Vol XIV, No 3

# OBSERVATIONS ON FILARIASIS IN SOME AREAS IN BRITISH INDIA

## Part II.

BY

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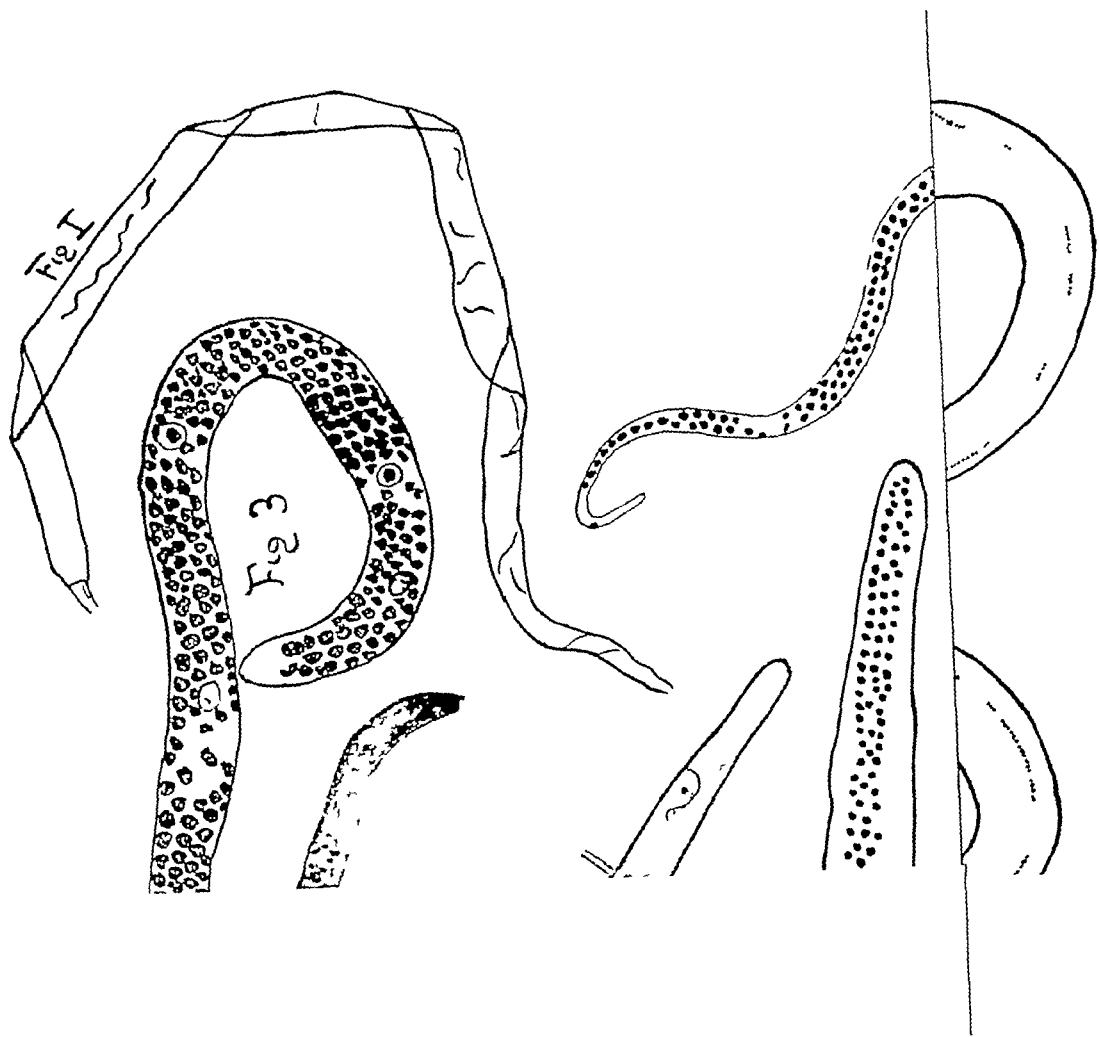
- I INTRODUCTION
- II MATERIAL AND TECHNIQUE
- III RESULTS OBTAINED IN FIELD STUDIES
  - (a) Filarial Incidence in the Areas of Bihar and Orissa
  - (b) Morphology of the Microfilariae
    - (i) Human Phase
    - (ii) Mosquito Phase
- IV DISCUSSION OF RESULTS
- V CONCLUSIONS

## I INTRODUCTION

THE investigation into filariasis was undertaken under the auspices of the Indian Research Fund Association, Simla, and covered a period from September 1927 to March 1928. Gaya was made the centre of the inquiry.

The object of the inquiry was, first to determine the species of *microfilariae* prevalent in the endemic areas in relation to the physical characters of the district, secondly, to observe the early clinical features associated with such species, thirdly, to study systematically the morphology of the microfilariae and fourthly, to investigate the life-cycle of the parasite in the invertebrate host.

A morphological study to determine the type species, the prevalence of the type species in relation to the physiographical conditions and to the clinical observations, and finally the study of the life-cycle in the invertebrate host, I considered to be the guiding points to arrive at a true picture of the filarial situation.



Thick smears were made from the cases showing microfilariae and the material was then brought to the field laboratory at Gaya, with a view to the study of the morphological details

The result of such a procedure has been the collection of filarial material in respect of the scheme. Endemic foci have also been mapped out from the standpoint of epidemiology and much information has been gathered regarding the prevalent species of the parasite

### *Technique*

*Blood film*—The technique of examining the material consisted in taking a measured quantity of peripheral blood (20 cmm), making thick smears, dehaemoglobinizing the blood and staining the films with a weak solution of Giemsa and haematoxylin on the approved lines

Wherever *intra vitam* staining was resorted to, it was done by Azure II in normal saline, strength 1 in 3,000

A routine method adopted was, to make camera-lucida drawings of the parasites found in each area, invariably under the same magnification. In this manner, the morphological differences, if any, were prominently brought to notice

The measurements were taken from the cephalic extremity (C E) to the centre or the end of the anatomical fixed point, as the case may be. The nomenclature of the anatomical fixed points adopted was, mainly after Fulleborn, viz —

C S—Cephalic space, a clear space without granules

N—Nerve ring, a transverse break in the continuity of body cells

Ex P—Excretory pore, V-spot

Ex Z—Excretory cell

I K—Innen korper—central viscus or a central matrix with a cellular mass

G<sup>1</sup> Z—Chief genital cell (Genital cells of Rodenwaldt)

A P—Anal pore, tail spot

L S Z—Last tail cell or caudal cell

S—Tail or caudal extremity

*Mosquito*—With a view to study the life-cycle in the invertebrate host, mosquitoes were caught in nature from the houses situated in known endemic areas. In order to keep them alive the following technique was adopted (see text Fig 1). The technique was kindly suggested to me by Captain P J Barraud of the Central Research Institute, Kasauli

1 Place the mosquitoes caught in nature in receptacles such as hurricane lamp glasses, the top and bottom of the glass being covered with mosquito netting (small mesh)

2 Place the lamp glass containing the mosquitoes in a shallow wooden box lined with several thicknesses of white lint, the lint to be kept damp, and not allowed to become dry

3 On the top of the lamp glass place a strip of folded lint, about one inch wide when folded, this to be kept damp also

# EXPLANATION OF PLATE XVIII—(contd)

- Fig 11 Expt 21, a few hours, thickened sausage-shaped body  $120 \times 28$  M in the wall of the alimentary canal of mosquito, cells deeply stained into thickened mass, extremities, one broad, one narrow, showing one clear space, St intra-vit, Scale C
- „ 12 A and B Expt 23, A a few hours, cylindrical body,  $525 \times 50$  M, showing the oral extremity, intestinal canal in formation, body filled with cells, caudal extremity broad and rounded St intra-vit, Scale A B Part of A figure (cephalic extremity) magnified Scale B
- „ 13 Expt 16, 12th day, cylindrical body,  $300 \times 30$  M No mouth parts, no definite alimentary canal, only a suggestion, by grouping the cells in the formation of a canal The cylindrical body shows clear (dotted) and deeply stained (not dotted) spaces Scale B
- „ 14 Expt 16, 14 days, cylindrical body,  $420 \times 40$  M, cellular, large refractile spot at the tail end, mouth parts forming, oesophageal bulb visible, evidence in the formation of alimentary canal, specimen fresh Scale B
- „ 15 A and B Expt 23, A 3rd day, large cylindrical body,  $500 \times 30$  M, 2 clear spaces encircled by dotted lines at the cephalic extremity which is pointed The intestinal tract is deeply stained but no structure is visible St intra-vit, Scale B B Caudal extremity, dotted lines show the area of deep staining, Scale C
- „ 16 A, B and C Expt 23, A 3rd day, filariform body,  $800 \times 40$  M, St intra-vit, Scale A B same specimen showing the mouth parts, nerve ring and the oesophageal bulb Scale B C showing the rhomboidal cells lining the intestinal canal, about 20 cells could be distinctly counted in pairs on either side, St intra-vit, Scale B
- „ 17 A, B and C Expt 21, 3rd day, A filariform body, 1 mm in length Scale A Movements vigorous, with change of place B and C showing the cephalic and caudal ends respectively St intra-vit, Scale B
- „ 18 A, B and C Expt 25, a few hours, A body similar to above,  $900 \times 50$  M Scale A B and C Cephalic and caudal extremities respectively Scale B The dark colour shows the area of deep staining in the caudal extremity
- „ 19 A, B and C Expt 25, a few hours, A filariform body,  $1,225 \times 17$  M, very active St intra-vit, Scale A B and C Cephalic and caudal extremities respectively Scale D Movements are active accompanied by change of place

Figs 6 to 19 in the thoracic organ of the mosquito

and in whose blood microfilariæ were not detected In both tables, an analysis of the signs and symptoms is given

*Filarial Incidence in Relation to the Physiographical Conditions of an Area*

The areas showing serial Nos 1 to 9 in Table I-A, belonged to the district of Gaya, 10 and 11, to the district of Patna, and 12 to the town of Puri

The district of Gaya is essentially a rural area of about 5,000 square miles and along with the district of Patna forms a part of the right bank of the Ganges

The slope of the country is from south to north The southern portion of the district is hilly and forms the watershed of the rivers of Gaya

The northern portion is an alluvial plain through which the rivers course towards the Ganges in the north

The water-supply is from wells which are shallow There is considerable subsoil moisture as the rivers of Gaya bring an amount of gravel and fine sand during the floods and raise their own level when they reach the plains

The average maximum temperature rises to 105°F and the humidity averages 51 per cent of saturation The average rainfall is about 45 inches The relative humidity during the monsoons averages 84 to 87 per cent and falls lower in September

The total population is well over two millions of which 5 per cent live in the urban areas and the remainder congregate in about 8,000 villages.

The density of the population in the northern division of the district is 666 per square mile, e.g., Jehanabad, the maximum, and in the southern division it is 257 per square mile, e.g., Barachatti, the minimum

Rice is the chief cultivation of the district The town of Gaya like Puri is a great pilgrim centre, visited by pilgrims throughout the year, and more so during the monsoon months

Gaya and Puri towns are known to be endemic centres of filariasis and the mosquito nuisance is prevalent throughout the year

Bihar town in the Patna district shows identical insanitary conditions with Gaya

The other areas are rural, some are more cultivated than others, some are situated at the foot of the hills, as for instance Madanpur and Giryak, and the rest on the alluvial plains

The incidence of filariasis in all the twelve areas is given in the form of table (Table II), which is self explanatory

*Clinical Signs and Symptoms*

The clinical signs and symptoms are noted in their order of frequency

*Duration of illness*—As regards the duration of illness and the history of fever, one had to rely on the information given by the patient Although the information given may not be accurate, still it was trustworthy to a certain extent The affection of the genitals in a class of people who marry early, is a strong reason to remember the onset of illness and to seek the advice of a medical man



in the lactic acid as the tissues are liable to get disassociated. The specimens can be returned to saline-formalin, or mounted directly in Canada balsam.

### MORPHOLOGY

(See PLATE XIX)

The parasites are whitish in colour, and the majority of them are banded by a dark, loose and detachable ring, like a rubber band over a pencil.

The females which show the above characteristic measured from 38 to 45 mm in length and 1 6/2 mm in breadth at the oesophageal bulb. The loose dark chitinous ring, about 1.5 mm in breadth, is situated in the region of vulva (Fig 7). The caudal extremity is straight, pointed and partially or wholly sheathed by a loose sheath, the 'prepuce' (Fig 8, shown without sheath and Fig 15).

The males measure from 35—45 mm in length and 1.2—1.8 mm in breadth at the oesophageal bulb. On the whole, the males are smaller than the females in length. The caudal extremity is curved or reflected on the ventral surface and is encased in the loose sheath as in the case of the females (Fig 9, shown without sheath and Fig 10).

The cuticle in both the sexes show a very fine transverse striation and is partially reflected over the lips forming a well marked cephalic collarette (Figs 2, 3, 4 and 5).

*Mouth*—The oral aperture is surrounded by two large conical lips (Fig 5), measuring about  $150 \times 200$  microns and ornamented with two well defined teeth about 20—25 microns (Fig 6). The inner median tooth was not found to be tripartite as has been observed by Ortlepp (1922).

*Oesophagus*—The oesophageal tube is long, about 5.7 mm in length, possessing a single muscular bulb measuring about 600 microns, and terminates in the dilated chyle-intestine with a posterior lobed apparatus (Fig 1). The length of oesophagus is about 1/5th of the total length of the parasite.

*Female*—The caudal extremity of the female (Fig 8) presents a narrow finger-like process. The vulva opens about 15—20 mm from the oral extremity, in front of the middle of the body and is non-protuberant. It measures about 120 microns in diameter and leads into a thick walled straight vagina about 1.5 mm long and 100 microns thick (Fig 14).

The posterior part terminates in an egg-chamber. The ova are thick shelled. Those containing a fully developed embryo measure about  $45 \times 30$  microns in diameter (Fig 16, A, B and C). The anal opening is found at about the posterior half of the caudal extremity.

*Male*—The tail is elongate and often reflected on to the ventral surface (Fig 9). The ventral surface is ornamented with the longitudinal rows of rounded tubercles, interrupted by longitudinal curved ridges (Fig 10). The common cloaca is situated about 3.5 mm from the tip of the tail and measures about  $500 \times 475$  microns (Fig 11). There are 3 *pre-anal* sessile papillae in a row, the middle one of which is larger than the others. There are 3 pairs of *post-anal* sessile papillae situated one behind the other, more or less equal in size. Two additional pairs of ventral sessile papillae somewhat



## EXPLANATION OF PLATE XIX

- Fig 1 Anterior extremity showing the œsophagus and cervical collarette
- Figs 2, 3 and 4 Anterior extremity magnified showing the cephalic extremity and cephalic collarette from different aspects Fig 3 on the same scale as fig 2
- Fig 5 Showing two lateral lips with cephalic collarette
- „ 6 Showing teeth
- „ 7 Showing chitinous band around the female at the vulval region The band is split open
- „ 8 Caudal extremity of female, devoid of sheath
- „ 9 Caudal extremity of male, devoid of sheath Figs 7, 8 and 9 on the same scale
- „ 10 Caudal extremity of the male showing common cloaca, spicules, pedunculated and sessile papillæ, ornamentation and preputial sheath (partly free hand drawing)
- „ 11 Showing common cloaca in the male, details as in Fig 10
- „ 12 A and B Showing long and short spicules respectively
- „ 13 A and B Spicules at the spicular cell magnified
- „ 14 Showing vulva and thick vaginal canal containing ova
- „ 15 Caudal extremity of female with preputial sheath
- „ 16 A, B and C Ova from uterus in different stages of development

With regard to the history of fever, the patient complained of frequent attacks, with occasional remissions lasting for a considerable time. Along with the attacks of fever he often noticed an uncommon enlargement and turgidity of the hydrocele. This condition subsided in the course of time and was concomitant with the remission in the febrile state.

Such was the type of history one elicited from the cases showing the presence of hydrocele.

In Table I-A, 56 and 64 per cent, and in Table I-B, 49 and 59 per cent, gave a history of fever and showed the presence of hydrocele, respectively.

Cases showing enlargements of the lower extremity were of more frequent occurrence (33 per cent in Table I-B), than those having affections of the upper extremity (8 per cent in Table I-B).

Edema of a fugitive nature was noticed in one instance only.

The scrotal enlargements noticed varied in size. The enlargement of scrotum in the Bihar area never reached a size sufficient to show an enlargement through the dress or to interfere with the normal gait in walking. The percentage of affection was 10 in Table A and 14 in Table B.

The glands most frequently implicated were the inguinal. They reached a size which was easily palpable, were indurated and sometimes not moveable over the subjacent fascia (18 per cent in Table I-A and 6.5 per cent in Table I-B).

The component parts of the testes were found to be affected. The percentage of affection was 10 in Table I-A and 2 in Table I-B.

In one instance (at Bihar), the urine was found to be chylous. In this case the evidence was the presence of hydrocele, duration 3 years, occasional attacks of fever and pain in the body and extremities. The blood examination made at 10-30 p.m. showed microfilariae in the peripheral blood. The parasites were not detected in the chylous urine.

## (b) MORPHOLOGY OF *Microfilaria bancrofti*

### (1) *The Human Phase*

I have shown before (Korke, 1927), that in a rapid survey where many dry films are stained on general lines for a routine examination, the anatomical points most liable to be overlooked were the excretory cell and the genital cells, unless these organs happen to be a *strong feature* of the specimens.

In order to identify *Microfilaria bancrofti* on morphological grounds alone, the following points in particular were to be noted in the microfilaria:

- (a) The character of the cephalic armature, if any
- (b) The shape of the tail
- (c) The position of the last tail cell
- (d) The character of the area between L, S, Z and the caudal extremity, S
- (e) The presence of a sheath

Two additional points in the diagnosis are noted in the present paper:

- (f) Filarial periodicity

The singular feature in the life of the microfilaria is very well recognized. The following observation made at Gaya confirms the feature.





solution, whereas kala-azar blood hæmolyzes to a cloudy solution which on standing deposits a flocculent precipitate. This test was found quite satisfactory by Sia (1921), but later in the same year Sia and Hsien Wu showed that this test was the globulin test in another form.

A very similar phenomenon was previously reported by Hill (1913) who, when using a special diluent for the purpose of making leucocyte counts, observed that in cases of kala-azar the blood ran into great lumps which could only be broken up with great difficulty. This only occurred in cases of kala-azar. It seems probable that like Ray's hæmolytic test this result is due to the precipitation of globulin.

The characteristic of the formalin reaction in kala-azar is, as has been pointed out by Napier, the opacity of the gel, and not the mere jellyfication of the serum which occurs in many conditions. Considerable confusion has arisen from the fact that many practitioners have understood the test as meaning that a gel with some degree of opacity produced by formalin is diagnostic of kala-azar. This is not the case. This is analogous to the basing of a diagnosis of syphilis on a weakly positive Wassermann reaction, which we know to be totally unjustifiable.

To prevent this confusion Napier introduced the term 'Aldehyde reaction' to describe the white gel in kala-azar, hoping by the avoidance of the name 'formol gel' to emphasise the point that the opacity of the gel is the important part of the test. We shall return to this question later.

Three stages may be recognised in the formol gel reaction in kala-azar. First, the addition of formalin produces at once a faint whitish precipitate. Almost immediately jellyfication begins. Lastly, opacity commences to spread through the entire mass, and the serum becomes completely opaque in a very few minutes. These three stages may be more clearly distinguished in a 'slow motion picture' produced by diluting the kala-azar serum with increasing volumes of normal saline before the formalin is added. The power of gel formation is, however, soon lost on dilution, and is usually absent when an equal volume of saline has been added. If only half a volume of saline be added gel formation usually occurs.

If a fully positive kala-azar serum be diluted with 9 volumes of normal saline, we know that the globulin content of this diluted serum is less than that of undiluted normal serum. Nevertheless, addition of formalin produces a white precipitate in the diluted kala-azar serum, although gel formation does not occur in this dilution. Addition of formalin to the undiluted normal serum on the other hand produces little or no precipitate. Evidently then the precipitation which is the first stage of the reaction is not dependent upon the mere quantitative increase of the total globulin. Further, 'fortification' of the globulins of a normal serum by the addition of excess of euglobulin prepared by dialysis from another normal serum does not enable such a serum to react positively with formalin.

The dilution experiment above is of course incomplete as the dilution should be adjusted till the euglobulin content of the two solutions is equal. We are, however, leaving the quantitative estimations of euglobulin in normal and kala-azar sera for a subsequent paper.

counts in the mosquito phase (Table III, A and B) Similar counts are being made in other microfilariae to see if this point is one of specific importance

(11) *The Mosquito Phase* (see Plate XVIII)

The life-cycle of *Microfilaria bancrofti* was observed from the time of the ingestion of the parasite by the mosquito to the time when the parasite had developed into stages possessing a definite alimentary canal and situated in the thoracic muscles of the intermediate host

The mosquitoes were caught in nature from houses situated in the endemic centres of Gaya and Puri towns

They were kept alive at the laboratory by the method described under the heading 'technique' The technique was found to be satisfactory and the batches of mosquitoes were kept alive and active for over a fortnight at the laboratory temperature which ranged between maximum, dry, 32°C, wet, 21.5°C, minimum, dry, 19°C, wet, 15.5°C in the months of February and March

About six hurricane lamp-glass receptacles were kept at the laboratory in which the mosquitoes from a particular house and of a particular batch were introduced

Each day a certain number of mosquitoes was dissected in normal saline The stages of parasites were stained *intra vitam* by azure II The dried smears were then treated with distilled water to wash off the salt deposit, were dried and fixed in equal quantities of ether and alcohol Preparations were finally mounted in Canada balsam The results proved to be satisfactory

So long as the parasites retained the morphological characters of the human phase, staining by Giemsa or hæmatoxylin was found to be satisfactory, but it was not so when once the parasites had developed internal organs In the last instance they were stained *intra vitam* by azure II

The species of mosquitoes collected at Gaya in which the developmental stages were observed were without exception *Culex fatigans*\*

The mosquitoes from Puri were not identified but there is a strong presumption that they also belonged to the species of *C fatigans*

The total number of mosquitoes dissected at Puri was 101, out of which 12 showed developmental stages of *Microfilaria bancrofti* or 12 per cent

The total number of mosquitoes dissected at Gaya was 258, out of which 39 or 15 per cent showed developmental stages of *Microfilaria bancrofti*

On an average 14 per cent of mosquitoes were infected in nature from the above areas

*Morphology*—Two points served as a guide in determining the commencement of the mosquito phase The first one was the presence of microfilaria in the stomach blood of the mosquito possessing identical characters of *Microfilaria bancrofti* of the human phase The second point was the presence of a sheath

It was in this stage the parasite had entered the stomach of a mosquito and it was this stage that marked the commencement of the life-cycle in the invertebrate host

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\* I am indebted to Captain Barraud for the identification of the species



from another normal serum. This may result in the formation of a curdy yellow gel which does not at all resemble the typical kala-azar reaction. When euglobulin solution from a kala-azar case is mixed with a normal whole serum, either active or inactivated (by heating to 56°C for 30 minutes), the formol reaction is positive. Further, when the euglobulin solution from a kala-azar case is mixed with the pseudoglobulin plus albumin fraction of normal serum the formol reaction is also positive. On the other hand, if euglobulin solution from normal serum be mixed with pseudoglobulin and albumin from a kala-azar serum the formol test is negative.

It follows from these experiments that the formol reaction in kala-azar is of a double nature, one factor being specific and associated with the euglobulin fraction of the kala-azar serum, while the other is non-specific. The analogy of complement fixation naturally suggests itself. The secondary complementing factor is not, however, identical with complement as serologically understood, for inactivation of the whole serum does not prevent the formol reaction. These experiments also are some evidence that a special globulin may be present in kala-azar serum.

#### INFLUENCE OF SALTS AND OF UREA ON THE FORMOL GEL REACTION

We desired to ascertain if the presence of the Calcium-ion was essential to the reaction. We accordingly added to the serum neutral potassium oxalate, sodium oxalate and sodium citrate solutions respectively. We also worked with oxalated and citrated plasma. None of these salts had any influence on the formol reaction. In the case of ammonium oxalate, however, we found some degree of inhibition of the reaction. We were led, therefore, to test the action of other ammonium salts. These delayed or prevented the reaction. We next experimented with urea, and observed that strong aqueous solutions of urea completely prevent the formol gel reaction in kala-azar sera. This action of urea appears to be non-specific, as it is known that urea solutions have remarkable powers of dispersing colloidal aggregates. They are able to convert the gel into a sol (peptisation).

Strong aqueous solutions of urea can also redisperse the white gel formed by the addition of formalin to kala-azar serum.

#### EXPERIMENTS WITH UREA SOLUTIONS AND SERUM PROTEIN FRACTIONS OBTAINED BY DIALYSIS

In view of the inhibitory action of urea solutions on the formol gel reaction of whole kala-azar serum, experiments were carried out to test the influence of urea solutions on the formol gel reaction exhibited by protein fractions, also as to how far egg albumin solutions would give these reactions.

The results are as follows —

- 1 Kala-azar euglobulin + formalin = negative as already seen
- 2 Kala-azar euglobulin + urea + formalin = curdy precipitate with incomplete gel.

Experiment numbers	Mosquito numbers collected	Place and date of collection	Date of observation	Laboratory temperature, C	
				Dry	Wet
14	10	Police Lines, Gaya , 22-2-28	23-2-28	26	19.5
15	15	Jail Quarters, Gaya , 17-2-28	20-2-28	24	17.5
16	8	Staff Quarters, Gaya , 22-2-28	24-2-28 27-2-28	25.5 27	19 19
			29-2-28	28.5	20
			2-3-28	29	21.5
			5-3-28	31	20.5
			7-3-28	28	15.5
17	9	Gayana 1 Bigha, Gaya , 23-2-28	24-2-28 25-2-28	25.5 25.5	19 19
			28-2-28	27.5	19.5
18	20	Godavari, Gaya , 26-2-28	27-2-28 1-3-28	27 28	19 21
			2-3-28	29	21.5
19	10	Nadraganj, Gaya , 27-2-28	27-2-28 28-2-28	27 27.5	19 19.5
20	3	Laboratory, Gaya , 29-2-28	29-2-28	28.5	20
21	24	Old town, Gaya , 29-2-28	29-2-28 3-3-28	28.5 32	20 21.5
22	38	Nadraganj, Gaya , 29-2-28	29-2-28 1-3-28	28.5 28	20 21
			3-3-28	32	21.5
23	43	Godavari, Gaya , 2-3-28	2-3-28 5-3-28	29 31	21.5 20.5
			7-3-28	28	15.5
24	7	Nadraganj, Gaya , 5-3-28	5-3-28 7-3-28	31 28	20.5 15.5
25	24	Ramsagar, Gaya , 7-3-28	7-3-28	28	15.5
26	12	Ramsagar, Gaya , 9-3-28	9-3-28 10-3-28	28 28	16 16

test outlast the point at which the globulin/albumin ratio, to be discussed later, is normal

We may here note that when the globulin test is performed on a serum of a fully treated case with a normal globulin/albumin ratio, precipitation is very slight at first, but after about half a minute it suddenly increases to a maximum. This phenomenon is a good deal less marked when serum from an untreated case is tested.

#### HYDROGEN-ION CONCENTRATION AND ISO-ELECTRIC POINT

We have examined the H-ion concentration of a number of fully positive kala-azar sera. We find the H-ion concentration of the blood serum to be 7.40 (mean of 23 observations) as against 7.37 in normal serum (mean of 31 observations). The observations were carried out by the method of Hastings and Sendroy (1924).

This indicates a slight movement in kala-azar towards the alkaline side. Observations on the iso-electric point by Sørensen's method showed 4.30 for kala-azar serum as against 4.73 for normal serum. This raising of the pH and lowering of the iso-electric point which are found in kala-azar are probably associated with the marked increase in the serum globulin which occurs in that disease. As to what changes, if any, are produced in the serum globulin by artificial alterations of the pH is a question we will leave for the present.

#### SERUM PROTEIN ESTIMATIONS

We next proceeded to estimate quantitatively the protein constituents of kala-azar and normal sera. The method used was the refractometric method of Robertson. In each serum examined the chemical processes were carried out in duplicate, and the refractive index of each serum fraction represents the mean of three refractometric observations. The results for one serum, both chemical and refractometric, were always in very close agreement. We have now had a very considerable experience in the use of the dipping refractometer, and we give below some of our latest figures —

TABLE I

*Serum of untreated kala-azar cases (Indians)*

Total protein (in grams per 100 c.c. of serum)	Globulin	Albumin	Globulin/albumin ratio
7.918	5.895	2.023	2.906
7.194	4.716	2.478	1.903
8.173	5.078	3.095	1.640
7.561	4.126	3.435	1.201
8.173	4.738	3.435	1.379
9.666	5.576	4.090	1.363
10.173	6.524	3.649	1.790

These conditions in nature, I consider, are intimately related with the prevalence and predominance of a particular species of mosquito in a particular area

It is not sufficient merely to know what species of mosquito are prevalent in an area but to gain further knowledge on the factors which are responsible for the breeding and upkeep of the species in luxuriance

This point has a two-fold interest. Filariasis is a disease in which condition the intermediary host is distributed over a wide number of species of the mosquito which vary according to the physiographical conditions in the different countries

In India itself, the life-cycle of *bancrofti* has been observed in *Anopheles rossii*, but what mosquito, whether this, or *Culex fatigans*, acts as an intermediary host *par excellence* is a point which needs investigation

Another point one has to take into consideration is the so-called 'human carrier' of filaria. The results of the investigation have shown that about 10 per cent of the agricultural population over a widely distributed area harbour *Microfilaria bancrofti* without showing any evidence of the disease. This evidence has to be sifted from a scientific point of view

There is a great probability that a direct relation exists between a species of mosquito and the pathogenicity of *bancrofti*.

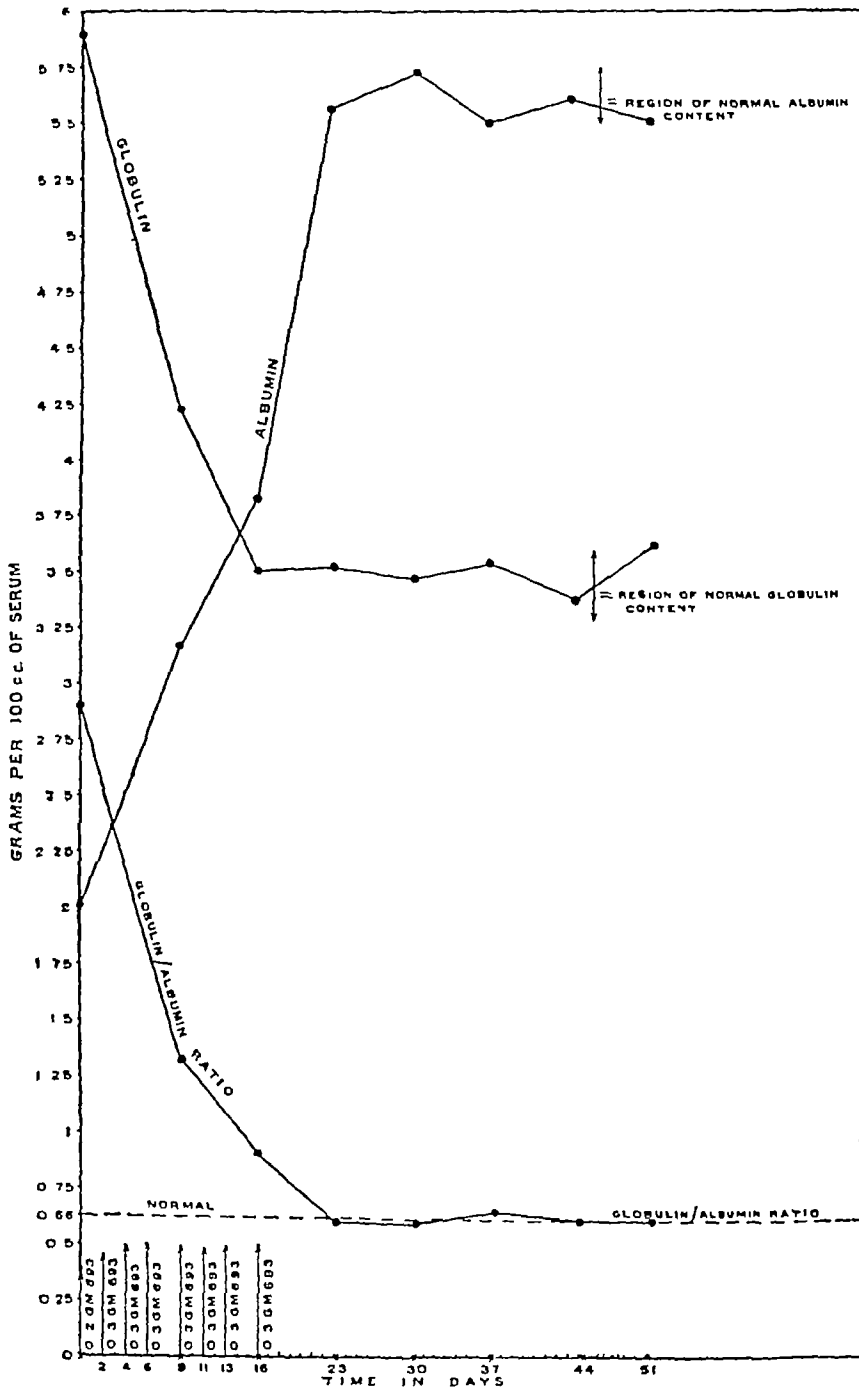
Evidence on these points in the present investigation has so far shown, that in a paddy cultivation area where the predominant species of mosquito is *Culex fatigans*, 25 per cent of cases showed *bancrofti* in the peripheral blood of the agricultural population *with pathogenic effects*, and 10 per cent of cases showed *bancrofti without such effects*

## V CONCLUSIONS

- 1 There is a considerable prevalence of filariasis in the Bihar and Orissa area
- 2 The evidence of filariasis was uniform throughout in an area cultivated chiefly with paddy
- 3 Twenty-five per cent of cases showed microfilariae in the peripheral blood where filariasis *was suspected* on clinical grounds
- 4 Ten per cent of cases showed microfilariae in the peripheral blood where filariasis *was not suspected* on clinical grounds
- 5 The microfilariae were detected most readily during the night time
- 6 A notable clinical feature was the affection of the genitals
- 7 The predominant species was *Microfilaria bancrofti*
- 8 There appears to be a definite relation between the total number of granules and the length of the *Microfilaria bancrofti* in microns, both in the human and mosquito phases
- 9 The life-cycle of *Microfilaria bancrofti* has been studied in *Culex fatigans*, the prevalent species of mosquito in the Gaya district
- 10 The developmental stages were observed from the time of the ingestion of the parasite by the mosquito up to the time when the parasite

GRAPH A

To show the effect of treatment on the globulin and albumin content of the blood serum in kala-azar



The arrows at the left hand bottom corner denote the treatment given and the days on which it was given

The first injection was given on the same day as, *but after*, the proteins were estimated Patient discharged from hospital 'clinically cured' on the 44th day

It will be seen that the albumin rose very rapidly from 2.0 to 5.5 gms per 100 cc of serum, the globulin equally rapidly falling from 6.0 to 3.5 gms per



of total globulin or the absolute decrease of albumin, both of which occur in kala-azar. Possibly the albumin is used up in some way in connection with the formation of a new globulin. It may be that the smaller albumin molecule undergoes some kind of aggregation with other colloids to form the larger globulin complex as suggested by Mayer. This question is at present quite obscure.

#### POSSIBLE APPLICATION OF THE GLOBULIN/ALBUMIN RATIO.

The treatment of a case of kala-azar by eight injections of von Heyden's compound 693 produces a complete reversal in the proportions of the serum proteins in three weeks, and the effect of the first injection is quite extraordinary. It is not impossible that this tremendous change in the serum proteins is the central fact of the cure, and that the destruction and disappearance of the parasites may possibly prove to be of only secondary importance.

While the number of cases under treatment submitted to this globulin/albumin ratio test is so far small, yet it is evident that the fall in the ratio which is at first rapid, later more gradual, which in a favourable case forms a smooth curve and is associated with progressive clinical improvement, and which is found to be normal (0.66) when a fixed amount of specific antimony treatment based on clinical experience has been given, represents a definite phase of the disease.

Study of the few cases so far examined indicates that the globulin/albumin ratio is not a mere replica of the general clinical condition of the patient. It is apparently something much more fundamental. For example, in one of our cases the ratio increased without treatment, afterwards to fall to normal when treated by amino-stiburea and later by 693, as shown in the subjoined Graph B.

These marked alterations in the ratio occurred without any conspicuous change in the clinical condition of the patient which showed little sign of improvement. It is noteworthy that this patient had previously received a course of treatment which had proved unsuccessful.

We also give below the globulin/albumin ratio graphs (Graph C) of two cases of our series which exhibited no particular clinical differences, both of which progressed satisfactorily to clinical cure.

It will be seen that their ratio curves are very different, that of case 1 being a smooth curve, while that of case 2 is spiky. Here again we find that intensive previous treatment (45 injections of urea-stibamine) had failed to cure case 2, while case 1 had received no previous treatment. Had the history not been known to the clinician, the spiky curve of case 2 would have been the only indication that it was of a resistant nature.

We have also had one case in which the ratio was normal without treatment. It was a very mild case, without fever for over two weeks, which in the opinion of Dr. Napier might possibly have been undergoing spontaneous cure. This case is also of interest as showing that a normal ratio is not further reduced by specific antimony treatment. We give below the graph of this case (Graph D).

We also show below two graphs (Graphs E and F) of two normally reacting cases.

## EXPLANATION OF PLATE XVIII

### THE MORPHOLOGICAL DETAILS OF *Microfilaria bancrofti* IN THE MOSQUITO PHASE

(The experiments mentioned below refer to those recorded in Table IV)

Fig 1 Expt 9, age, few hours, showing empty sheath of *bancrofti* (Human phase), about  $260 \times 8$  M, staining hæmatoxylin, Scale C

„ 2 Expt 9, age, few hours, showing *bancrofti* without sheath  $208 \times 5$  M, granules 258, specimen somewhat shrivelled, St hæmat, Scale D

„ 3 Expt 24, 2nd day, showing *bancrofti* without sheath,  $190 \times 10$  M, undergoing change Specimen has become thicker and stouter, granules large, thick, coarse and fill the whole body cavity Gaps of N, Ex P, and I K are only visible, area of I K finely dotted with red granules, S, a clear space, slightly stained red, St Giemsa, Scale D

„ 4 Expt 16, 5th day, showing *bancrofti* without sheath,  $180 \times 5$  M, undergoing change, granules have become thin, small and 'peppery,' possibly a degenerative change, anatomical points N, Ex P and A P show cluster of granules, St hæmat, Scale D

„ 5 Expt 6, a few hours, showing *bancrofti* without sheath,  $315 \times 8$  M, undergoing change, granules (about 340) are large, distinct and have a definite tendency to line the cuticular wall and showing canalization of the area, cuticle striated, Ex Z, I K and GIZ not distinguishable, St Giemsa, Scale D

Figs 1 to 5 in the alimentary tract of *C fatigans* The movements are sluggish, not accompanied by any change of place

„ 6 Expt 25, a few hours, showing cylindrical and sausage-shaped bodies  $100|175 \times 25$  M in the thoracic muscle of mosquito, St intra-vit azure II, Scale A

„ 7 Expt 16, 9th day, showing cylindrical body,  $150 \times 15$  M, with the remnant of tail, the whole body is uniformly and deeply stained, showing fine, evenly distributed granules—possibly a degenerative change, leaving out a clear area which would correspond to Ex P St intra-vit, Scale B Movements tail, are sluggish

„ 8 Expt 23, a few hours, body like in Fig 7,  $190 \times 28$  M, but showing distinct fine granules, St Giemsa, Scale B

„ 9 Expt 16, 9th day, body like in Fig 8,  $190 \times 22$  M, but showing distinct alimentary canal (deeply stained) with remnant of cells at the cephalic extremity There is a clear space at the anal area St intra-vit, Scale B

„ 10 Expt 16, 9th day, body like in Fig 9,  $190 \times 20$  M, presence of alimentary canal, deeply stained, intra-vit, Scale B

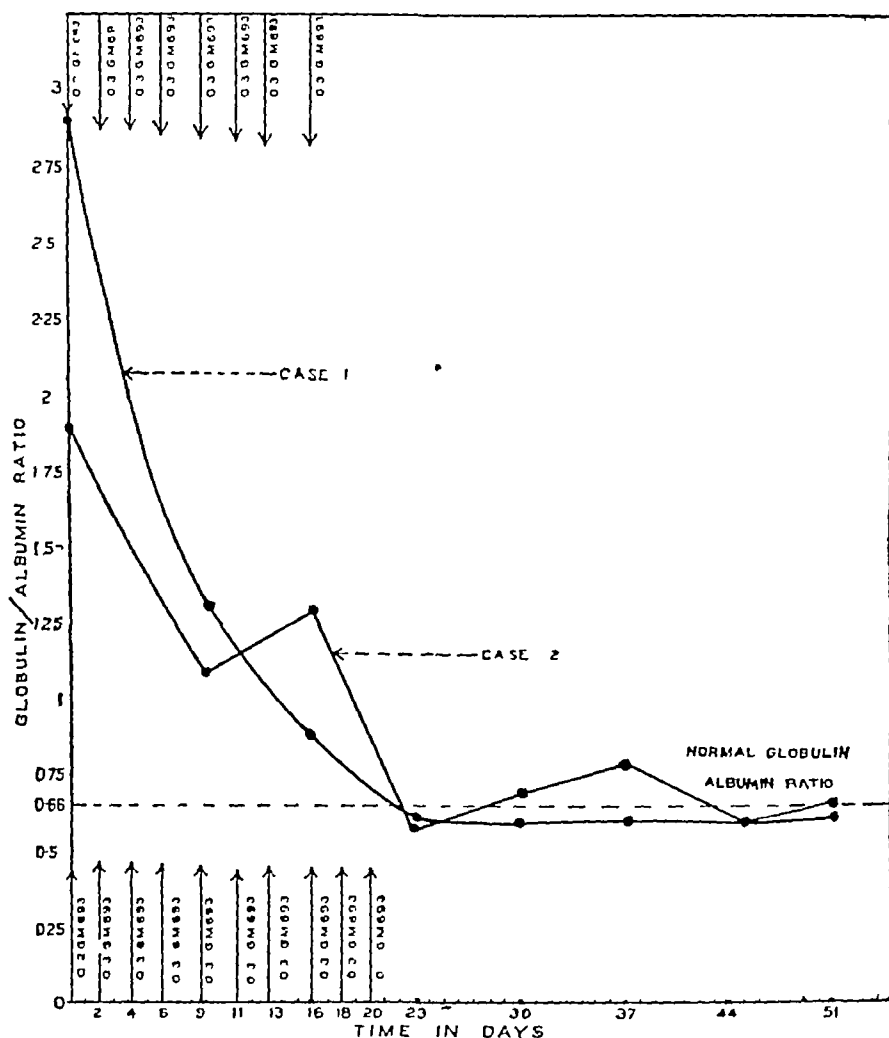


2 The formol gel reaction in kala-azar is therefore analogous to a complement fixation reaction, though the non-specific factor is not identical with complement, as it is not destroyed by inactivation

3 Euglobulin prepared by dialysis from normal serum will not yield this reaction These results therefore suggest that a special euglobulin is present in kala-azar, though we cannot at present exclude the possibility that the reacting

### GRAPH C

*To show the effect of treatment on the globulin/albumin ratio of two cases of kala-azar*



The first case shows a smooth curve The second case shows a decided tendency to relapse in the ratio

The upper arrows indicate the treatment of the first case, and the lower arrows that of the second case

The first injection was given on the same day as, *but after*, the first protein estimations were done.

# REVISION OF THE TYPE SPECIES OF VON LINSTOW IN INDIA.

PHYSALOPTERA PRÆPUTIALIS (LINSTOW, 1889)  
SYN CHLAMYDONEMA FELINEUM (HEGT, 1910)

BY

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[Received for publication, April 2, 1928]

## INTRODUCTION

THE study of the nematode parasites, whose genera have been found to be parasitic to man, is considered essential at the present state of our knowledge in India

*Physaloptera caucasica* Von Linstow, 1902, has been observed in the intestine of man in the Caucasus and *P mordens* Leiper, 1907, has been found in several cases in Nyasaland and Portuguese East Africa, in the œsophagus, stomach and small intestine of man. These are large worms resembling an immature *Ascaris lumbricoides*

The genus *physaloptera* has been widely distributed over Amphibia, Reptilia, Aves and Mammalia, but the present communication is concerned only with the parasite common in carnivores such as cat, leopard and panther

## MATERIAL

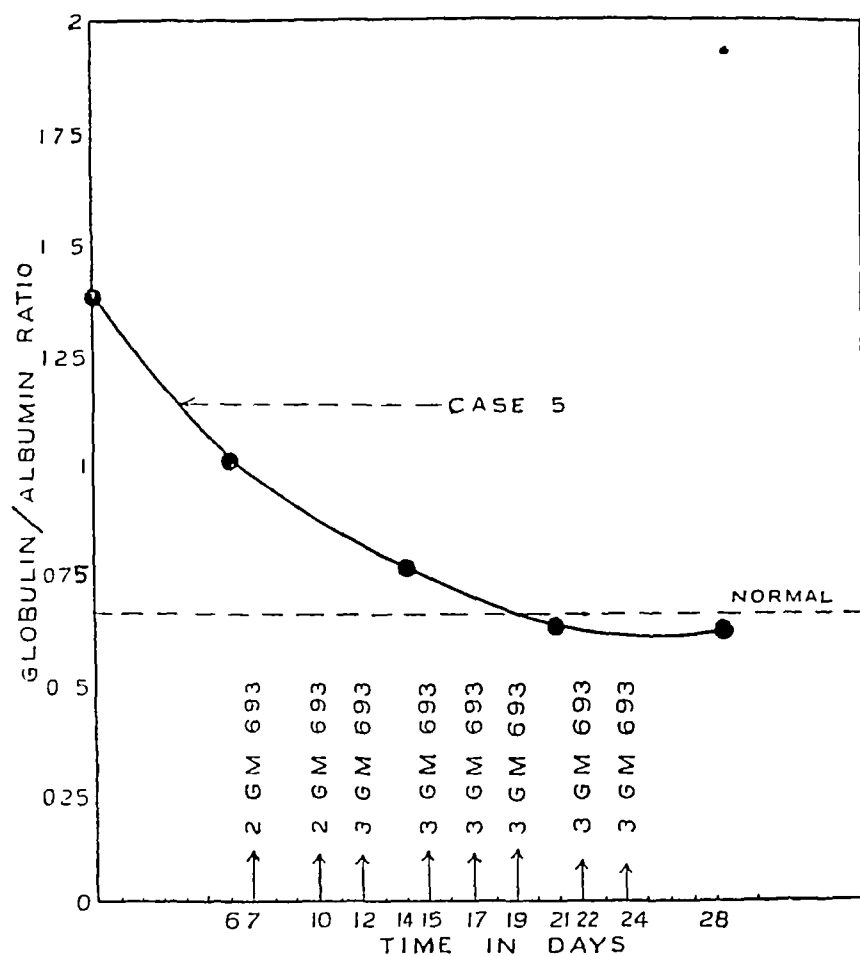
This was obtained from the stomach of a wild panther trapped and shot at Kasauli, Simla Hills, in 1921. The worms were cleaned, fixed, labelled and stocked for future examination

Two methods of clearing were employed. The one consisted in preserving the specimens in 'saline formalin,' i.e., 3 to 5 per cent of formalin in normal saline and clearing in pure lactic acid (Method, Ecole Nationale Vétérinaire D'alfort, Paris, personal notes), and the other in preserving the specimens in 70 per cent alcohol, transferring them to 90 per cent alcohol and clearing in pure beechwood creosote

Both methods were satisfactory. The advantage of the first method lies in the fact that it dispenses with alcohol altogether and the specimens are rapidly cleared. Care should, however, be taken that the specimens are not kept too long

5 Treatment of a well established case of kala-azar with von Heyden's compound 693 results in an immediate and enormous change in the serum proteins, the globulin rapidly diminishing and the albumin rapidly increasing until, after eight injections of 693 in three weeks, the globulin/albumin ratio has fallen to normal. When this point is reached, the serum is still abnormal in other respects, for the globulin precipitation test is still positive, and formalin can still produce a semi-opaque gel. The reduction of the globulin/albumin ratio to normal is thus the first stage towards normality of the serum.

GRAPH F



6 The curve showing the fall in the globulin/albumin ratio under treatment is, in a favourable case, a perfectly smooth one. One case which intensive previous treatment had failed to cure has shown an irregular graph, the curve tending eventually to reach the normal base line (0.66).

7 The information so far available suggests that we have in these protein graphs a serological control by which the power of any particular drug in kala-azar may be evaluated. This will be more closely examined later.

larger than the above are situated further down the tail (Fig 10) The four pairs of pedunculated papillæ are thick, measure about 450 microns Two pairs are *pre-anal* and two are *post-anal* (Fig 10)

The spicules are unequal, curved and terminate in a point The right spicule is short and thick and measures about 425—700 microns in length (Figs 12B and 13B) The left spicule is long and slender and measures about 1,100 microns in length (Figs 12A and 13A)

### CLASSIFICATION (after Yorke and Maplestone)

Family, PHYSALOPTERIDÆ Leiper, 1908

*Definition*—SPIRUROIDÆ mouth with large simple triangular lateral lips armed with one or more teeth, cuticle reflected forwards over the lips to form a cephalic collarette, cutaneous cordons or epaulettes absent, usually without a vestibule, œsophagus divided into two portions Male caudal alæ well-developed, usually meeting ventrally in front of the cloaca, and supported by long costiform papillæ

Subfamily, PHYSALOPTERINÆ Railliet, 1893

*Definition*—PHYSALOPTERIDÆ with the characters of the family

#### Key to Genera

- |   |                     |
|---|---------------------|
| 1 Cervical papillæ modified into large crescentic tooth-like structures                     | <i>Streptocara</i>  |
| Cervical papillæ simple   | 2                   |
| 2 Without a cephalic collarette, vestibule present  | <i>Thubumæa</i>     |
| With a cephalic collarette, vestibule absent  | 3                   |
| 3 Male with caudal alæ not meeting ventrally in front of cloaca, vulva near anus            | <i>Proleptus</i>    |
| Male with caudal alæ meeting ventrally in front of cloaca, vulva in front of middle of body | 4                   |
| 4 With a prepuce-like sheath over the posterior end of body                                 | <i>Chlamydonema</i> |
| Without a prepuce-like sheath over the posterior end of body                                | <i>Physaloptera</i> |

The morphological characters described, agree with *Physaloptera præputialis* the type species of Von Linstow, 1889 The generic characters are those of *Chlamydonema* The specimens therefore belonged to the type species *Chlamydonema præputiale* (Linstow, 1889) Syn *Physaloptera præputialis* (Linstow, 1889) \* *Chlamydonema felineum* (Hegt, 1910)

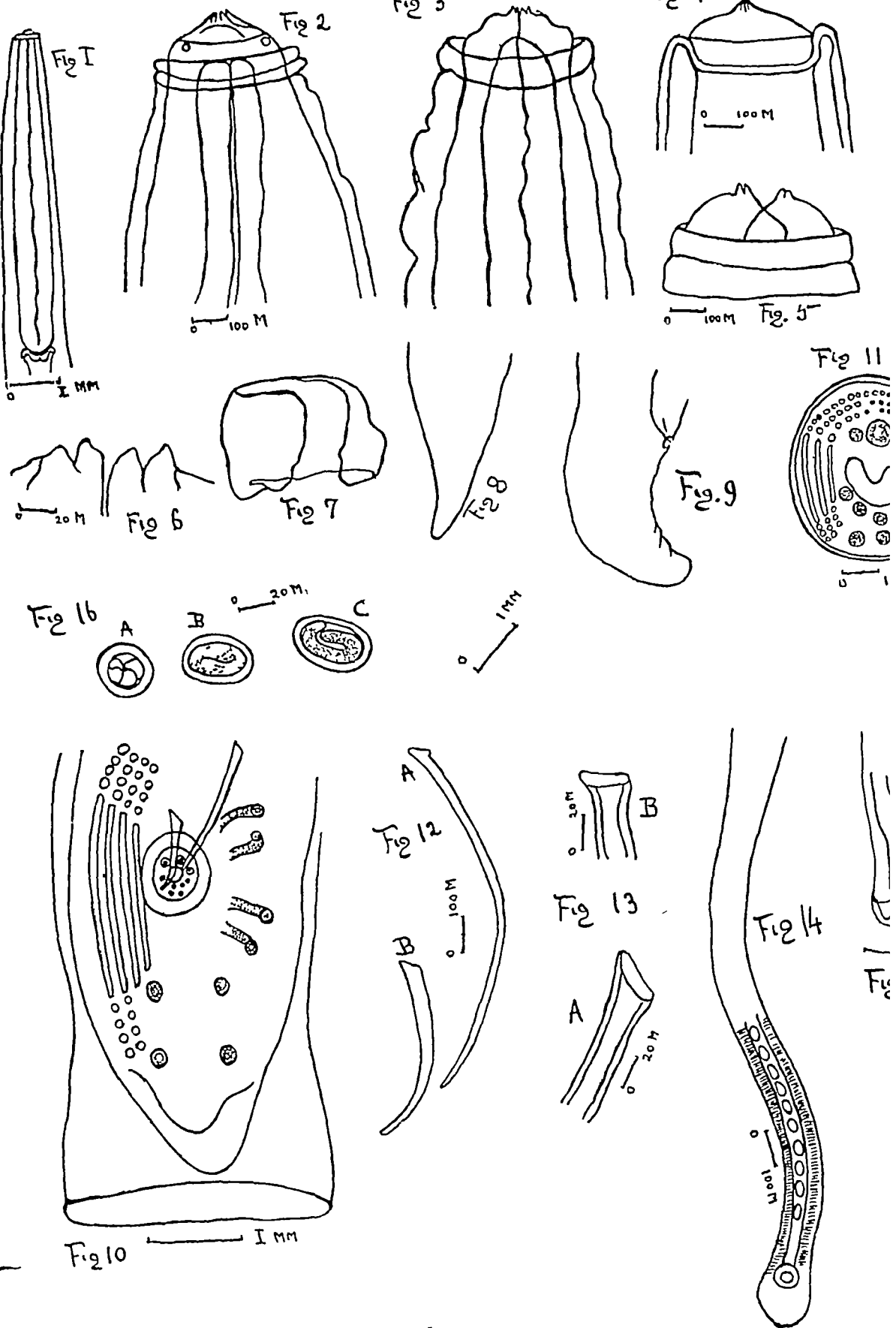
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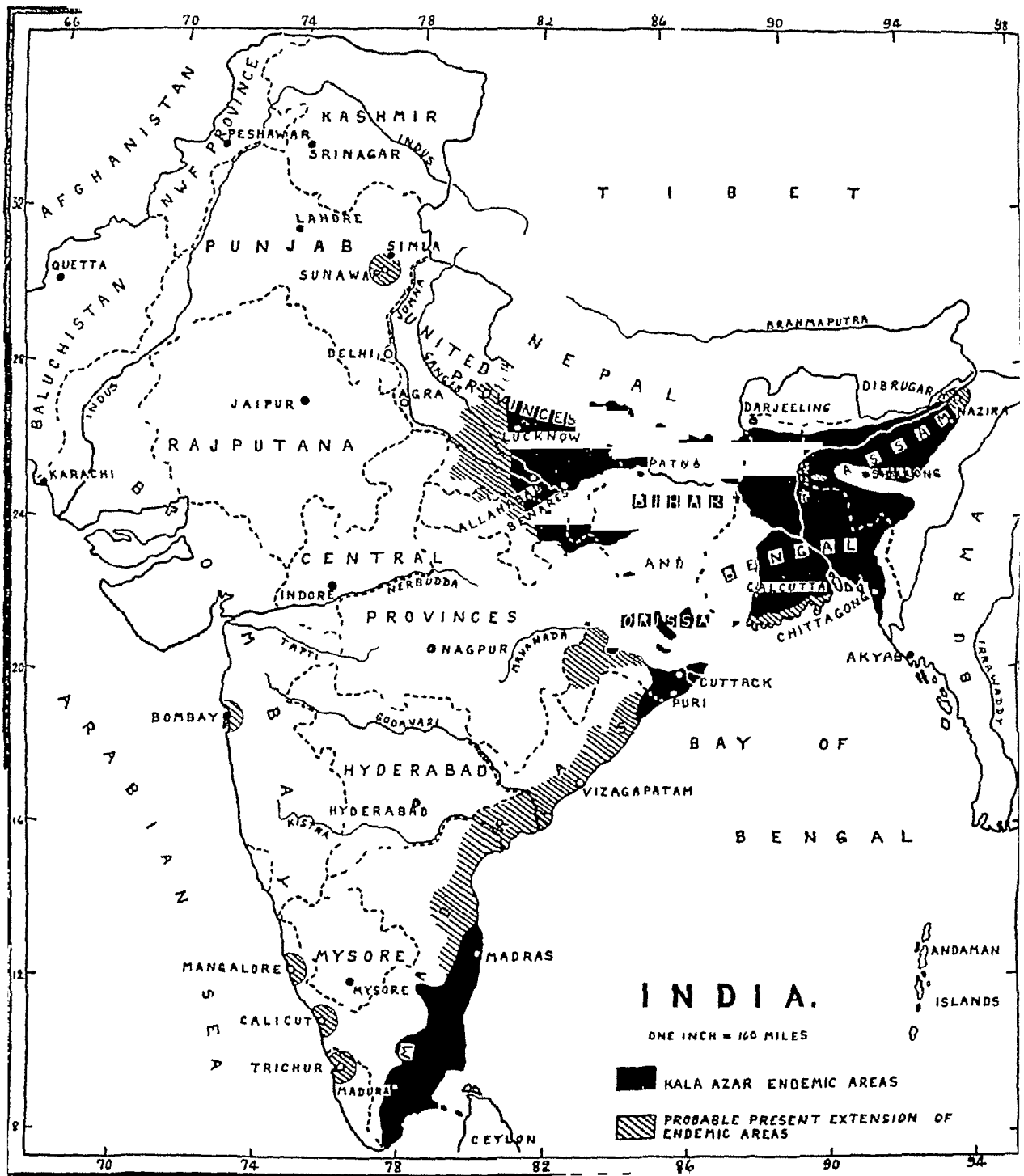
\* Yorke and Maplestone (1926) give the specific name as '*felineum*', while Ortlepp (1922), whose work on the genus *Physaloptera* is well known, gives the specific name as *felineus*

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# PLATE XIX



# PLATE XX



DISTRIBUTION OF KALA-AZAR IN INDIA

# SERUM CHANGES IN KALA-AZAR

BY

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## INTRODUCTORY

THE present paper is a report on experiments conducted to investigate the nature of certain blood changes in kala-azar. We first of all examined the formal gel (aldehyde) reaction. The observation, that when a drop of formalin is added to 1 c.c. of serum from a case of kala-azar an opaque white gel rapidly forms, was first made by Spackman (1921). This phenomenon was further studied by Napier (1921), (1922), (1923), who thoroughly tested it, and utilised it clinically in the diagnosis of kala-azar. A month after Napier's first announcement Fox and Mackie (1921) who had been working independently also reported a similar series of tests.

Brahmachari (1917) observed that when a few drops of a kala-azar serum are added to excess of distilled water a copious precipitate is formed, while normal serum produces only a very faint cloud. He considered this reaction to be due to the precipitation of a globulin-like substance, and the test was termed the globulin test. He also found that the precipitate had some anti-complementary properties. He further noted that the smaller the quantity of water added the more specific was the test. This is no doubt due to the fact that in kala-azar the euglobulin is greater in quantity than in any other disease, as the higher the euglobulin content of the serum the smaller will be the volume of water necessary to precipitate it.

Ray (1921) described a hæmolytic test depending on the addition of two drops of blood to ten volumes of water, when normal blood hæmolyzes to a clear



detailed account of distribution, but a few remarks must be made to indicate its scope and limitations. The criterion made use of in determining the presence or absence of kala-azar in any particular region is the occurrence of cases of the disease which have indubitably had their origin in the district under consideration. So long as the extreme limits of the incubation period of kala-azar are unknown this distinction can never be absolute in the case of persons who have at some time or other left the region under consideration but, as most of our information is based on numbers of cases in persons who have never left the area in which they contracted the disease, the data may be considered fairly reliable.

The most characteristic feature in the distribution appears to be the limitation of the disease to low-lying land, especially the broad alluvial valleys of the larger rivers, and the lower ranges of the hills bordering these. It seems probable that further study may result in a considerable expansion of the endemic areas beyond those now marked on the map.

## II THE FORMS OF PREVALENCE OF KALA-AZAR SEEN IN INDIA

### *Endemic Kala-azar*

Over the whole of the endemic areas marked on the map the disease may be said to be always present. The extent of this endemic prevalence has only been revealed within the last ten years as a result of the interest and alarm aroused by the epidemic which commenced about 1917 and is only now coming to an end. The research stimulated by the onset of this epidemic has shown that over practically the whole of Bengal, Bihar and Orissa and Assam cases of kala-azar can at any time be found. The same holds good, to an extent as yet not accurately delimited, of the United Provinces and Madras.

During the inter-epidemic periods of quiescence this sprinkling of scattered cases suffices to keep alive a smouldering fire of infection ready, when conditions become favourable, to produce a fresh conflagration in the form of an epidemic.

### *Epidemic Kala-azar*

This form of the disease is most typically seen in India, especially in Assam and Bengal. Although there is some evidence that epidemic outbreaks of kala-azar have occurred in the endemic areas of the disease in China, it is in India only that we have any definite information of virulent and widespread epidemics. How virulent these epidemics may be, and the terrible havoc they may cause in districts ravaged by them, are a matter of recent history in the province of Assam, but only those who have seen the complete destruction of whole villages within a period of a few years, and the consequent loss of productivity of large areas of cultivable land, can have any idea of the social and economic chaos which such a fatal disease may cause, when no treatment for it exists. Happily, owing to the introduction of efficient treatment, such a state of things as existed in previous epidemics can never again obtain.

We give below a very brief résumé of the known history of kala-azar epidemics in India, since a knowledge of these is essential to an understanding of the importance of the problem of kala-azar to India.

## DIALYSIS OF KALA-AZAR SERUM \*

When kala-azar serum is dialysed in a collodion sac against running distilled water, a heavy precipitate of euglobulin rapidly falls, very different to normal serum which only deposits its euglobulin slowly and in much smaller amount. Without quantitative analysis it is obvious, as has been found by all observers, that the euglobulin in kala-azar is much increased.

For our purpose, dialysis was taken to be complete when a drop of the clear supernatant fluid withdrawn from the dialysing sac produced no precipitate when added to a test tube full of distilled water (any euglobulin remaining in solution will be precipitated by a large excess of distilled water). When this stage of dialysis is reached, usually two to three days, the contents of the dialysing sac were poured into a centrifuge tube, and thoroughly centrifuged. The supernatant fluid containing, as regards proteins, the albumin and pseudoglobulin was pipetted off. The precipitated euglobulin was thoroughly washed several times with distilled water, and centrifuged after each washing. After pipetting off the wash water, the euglobulin was then dissolved in the minimal volume of 5 per cent sodium chloride solution. It was then found that addition of formalin to the pseudoglobulin plus the albumin fraction produced no visible change. Similarly, addition of formalin to the euglobulin fraction produced no visible change. If, however, the two solutions be mixed, and then a drop of formalin be added, the usual white gel forms. The same result follows if formalin be added separately to each of the two protein fractions, the two solutions being subsequently mixed. Gel formation with these protein fractions is a somewhat slow process, and is seldom complete under one hour. It usually takes several hours, and in some cases the reaction may be fully positive after six to twelve hours. The delay in the reaction is no doubt due, in part at least, to the dilution which the serum constituents have undergone during dialysis. Mixtures of the euglobulin solution from one case of kala-azar with the pseudoglobulin plus albumin fraction from another kala-azar case give the positive formol reaction in the usual way.

## DIALYSIS OF NORMAL SERUM

Normal serum was then dialysed, and its euglobulin after washing was dissolved in 5 per cent sodium chloride solution in exactly the same way. It was then found that the dissolved euglobulin, the pseudoglobulin and albumin fraction, and mixtures of these two protein fractions react negatively to formalin in every case. In comparing normal sera with kala-azar sera due allowance was made for the excess of euglobulin occurring in the latter condition by using euglobulin solutions of the same strength in the two cases, as measured by the opacity produced in the water precipitation (globulin) test. The negative result in normal serum is, as already stated, unaffected by artificially increasing the euglobulin in a normal serum by the addition of excess of euglobulin precipitated by dialysis.

\* The term 'kala-azar serum' as used in this paper means in every case serum obtained from definitely diagnosed cases of kala-azar, in which the Leishman-Donovan body was present.

and only a comparatively immune population survives. As a new generation arises it affords an abundance of susceptible material as fuel for a fresh epidemic fire.

Another consideration which seems fairly evident, and which may again be connected with the question of an acquired immunity, is that when kala-azar in epidemic form reaches an area previously unaffected by the disease, it seems to assume a specially virulent and fulminating form. A study of the disease in Assam leaves one in little doubt that in the Brahmaputra valley it is definitely a new disease of the last 100 years, and it is in this area that it has caused the most terrible ravages, as we have seen in an earlier part of this paper. This factor of the greater virulence of a newly introduced disease is, of course, not peculiar to kala-azar and is seen in other diseases even when not protozoal in origin.

A third factor which has emerged from our study of epidemics of kala-azar is one for which we have, at present, no explanation to offer. This is the fact that when the edge of a kala-azar endemic area encroaches upon a hitherto uninfected area and introduces the disease in epidemic form, or when the disease in an old endemic area suddenly lights up into an epidemic outbreak, the epidemic is not limited to any one area in the country. Closely following in time the area where the fresh epidemic was first noticed, or even coincidently with it, we find all the smouldering fires which mark the remains of previous epidemics in other and often far distant areas burst into flame and a general conflagration results which involves all the areas now infected and those which had previously been so. This striking phenomenon implies, so far at least as this disease is concerned, some universal and far-reaching factor in the causation of epidemics of which we at present know nothing. This factor may have some relation to the bionomics of the parasite of kala-azar or of its intermediate or definitive hosts, or may be the result of the sum total of the inter-actions between all three, in any case the phenomenon is one which would well repay study by professional epidemiologists. It was well exemplified in the epidemic of kala-azar which has been in progress since 1917 and is only now coming to an end.

We have seen that since the time when more or less accurate records began to be kept, kala-azar had advanced eastwards up the Brahmaputra valley in a series of leaps, each leap representing an epidemic of kala-azar in the virgin soil of a non-immune population lying on the limits of the area affected by the epidemic immediately preceding. Thus the epidemic in Nowgong of the years 1891—1901 carried the disease eastwards to the borders of the Sibsagar subdivision where it continued to smoulder. From 1917 onwards a fresh epidemic commenced which now involved practically the whole of the Sibsagar subdivision. Coincidentally with the outbreak here, however, Nowgong itself, and all the country west of it which had previously been the seat of epidemic outbreaks, again showed the disease in an intense epidemic form. This simultaneous and widespread recrudescence of kala-azar was not even confined to Assam but appeared at the same time in Bengal and Bihar and Orissa, pointing to some far-reaching influence operative simultaneously over the whole of these regions.

- 3 Normal euglobulin + urea + formalin = opaque gel after 24 hours (gel not very firm)
- 4 Egg albumin + formalin = negative (gelatinous precipitate but no or slight gel)
- 5 Egg albumin + urea + formalin = positive (gel may be incomplete, yellowish and not thick white as in kala-azar)
- 6 Egg albumin + kala-azar euglobulin + formalin = positive (opaque white gel just as in kala-azar)
- 7 Egg albumin + kala-azar euglobulin + urea + formalin = negative
- 8 Egg albumin + normal euglobulin + formalin = no change at first but fairly hard opaque gel after 48 hours

In the above experiments reactions 6 and 7 appear to be the typical kala-azar reaction which is positive in the absence of urea but inhibited by its presence, the only difference being that egg albumin here functions in place of the pseudo-globulin and albumin fraction of the serum. More curious are the results 2, 3 and 5 in which urea appears able to determine a non-specific precipitation, but without or with incomplete gel formation.

#### DISCUSSION OF THE FORMALIN TEST

The white gel with formalin, while a very useful clinical test when properly applied, is not absolutely characteristic of kala-azar. Positive reactions have been reported in human schistosomiasis (Faust and Meleney, 1924). This fact does not impair the usefulness of the test in India where human schistosomiasis does not occur. Moderate degrees of opacity may be obtained in other conditions, e.g., malaria and leprosy. Kala-azar sera react negatively to acetaldehyde and chloral hydrate\* (saturated aqueous solution). It is not therefore a reaction general to aldehydes as a class. In treated cases and in early untreated cases of kala-azar semi-clear gels may be obtained with formalin. We do not think, therefore, that any very sharp line can be drawn between a clear gel and an opaque gel. It would rather seem to be a difference of degree only, as whether a gel is clear or opaque will depend upon the size of the particles formed, and upon their refractive index with reference to that of the medium in which they are formed. Nevertheless it is very desirable, as this test is used as a routine, that the name given to it should lay stress on the special appearances to be expected in kala-azar. For these reasons, we, with some diffidence, suggest the term 'formol leuco-gel reaction'.

Whether there be a special euglobulin in kala-azar or not, the formation of a white gel with formalin appears always to be associated with a high globulin content, possibly always with a high euglobulin content. Where the globulin excess is great, an opaque gel rapidly forms. Where the globulin excess is slight, a semi-opaque gel slowly forms, though in a case under treatment some degree of gel formation with formalin and a partially positive globulin precipitation

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\* Acetaldehyde produces after some hours a slight precipitate, but no gel. Chloral hydrate produces an immediate heavy precipitate which after some hours becomes creamy, but there is no definite gel formation.

*Mottling of the skin*—‘On the skin of the thighs numerous small pale areas could be detected’

*Papular eruption*—‘It is common in cases of the disease especially in the advanced stages to find a scanty or profuse papular eruption about the thighs, Scarpa’s triangle, and the scrotum. Similar papules are less frequently seen on the trunk, arms and neck. Some of these appear to ulcerate and form small ulcers covered with a raised scab, which are evidently slow to heal and chronic in nature. An unulcerated papule from a case showed, in section, bodies in small numbers scattered through the dermis. It appears distinct from the scabies seen on the hands of many of the cases, and the papule examined showed a deeper lesion than in this condition’

It has been stated that dermal leishmanoid is not accompanied by any visceral infection as spleen puncture and cultures from the peripheral blood give negative results

Das Gupta(16) and Brahmachari and Dutt(6) have, however, obtained cultures from the peripheral blood and the latter has seen the condition in a patient still suffering from actual kala-azar. It should not be forgotten that the source of cultures supposed to be from the peripheral blood might easily, in this condition, be the skin itself

As most cases of dermal leishmanoid have a previous history of kala-azar there seems little doubt that the causative parasite is actually *Leishmania donovani*

From cultures made from a case Das Gupta(15) has produced a general infection in a mouse and Shortt, D’Silva and Swaminath(49) have shown that *Phlebotomus argentipes* fed on the lesions of a case became infected and showed a development of the parasite exactly similar to that seen when the flies are fed on cases of kala-azar. While neither of these experiments would prove the identity of the parasite with *L. donovani* they supply confirmatory evidence

### III THE PARASITE OF KALA-AZAR.

In 1903 Leishman(24) described certain bodies which he had recovered three years earlier from the spleen of a man who had died as the result of a long continued fever contracted in India. Later in the same year Donovan(17) obtained the same bodies by spleen puncture in a patient during life

In 1904 Rogers(31) succeeded in cultivating the parasite *in vitro* and established its position as a flagellate protozoon

Since that date the parasite has received various names according to varying conceptions of its zoological position but it may be considered to be a species of *Leptomonas* although, on account of its vertebrate habitat during one stage of its life-history, it has been raised to generic rank as *Leishmania*. We need not here enter into a detailed description of *L. donovani*, the parasite of Indian kala-azar, since this information can be obtained from already published work, but a bare outline may be given for the sake of completeness

The parasite of kala-azar, as it occurs in man or other vertebrates, is a small aflagellate sub-oval or spindle-shaped body, the *Leishman-Donovan* body,

TABLE II

*Normal serum (Indians)*

Total protein (in grams per 100 c.c of serum)	Globulin	Albumin	Globulin/albumin ratio
8 225	3 344	4 881	0 685
8 238	3 165	5 073	0 623
7 823	3 165	4 658	0 678
8 456	3 349	5 107	0 655
7 987	3 117	4 870	0 640
8 136	3 235	4 901	0 660
8 202	3 366	4 836	0 694
<hr/> Average 8 152	<hr/> 3 248	<hr/> 4 903	<hr/> 0 662

It will be evident from the above figures that while there is no very marked change in the total serum proteins in kala-azar, there is a profound alteration in the constituent parts, i.e., the globulin is greatly increased while the albumin is greatly decreased.

The blood change which results in the opaque gel when kala-azar serum is treated with formalin is slow in its development. Napier has reported that a fully positive reaction is seldom found under five months from the commencement of the disease. We therefore examined a series of six cases of kala-azar\* of varying severity in order to see the effect of specific antimony treatment on these various serum reactions. We have carried out quantitative protein estimations at approximately seven-day intervals for as long as the patients could be persuaded to remain under observation. These cases yielded very interesting findings. From the moment treatment is instituted, the serum proteins undergo a profound and immediate change, the albumin rapidly rising and the globulin rapidly falling until after eight injections in three weeks of von Heyden's compound 693, the globulin/albumin ratio, which in a well developed case is anything from 1.2 to 2.9, falls to 0.66 (normal).

Moreover this change is steady, in that it does not relapse after the cessation of treatment. This is interesting as it corresponds with the dosage of eight injections of 0.3 gm of von Heyden's compound 693 which Napier arrived at on clinical grounds. These changes in the absolute values of the serum globulin and albumin and of the globulin/albumin ratio produced by treatment of a typical well established case of kala-azar are shown in Graph A.

\* These were all definitely diagnosed cases in which the Leishman-Donovan body was present.

## V THE GENUS *Phlebotomus* AND KALA-AZAR

### Historical

The first worker to consider the possible association of the genus *Phlebotomus* with the transmission of kala-azar was probably Mackie(25) who made the statement 'The only insect which has given any return for work put into it is the sandfly, and I am of opinion that the relation of this insect to the disease would repay further investigation' Awati(2) in a list of biting insects found in dwelling houses in Assam mentions only one species of *Phlebotomus*, viz, *P. perturbans*

### *Phlebotomus argentipes* and Kala-azar

As pointed out by Shortt and Swaminath(48) there is little doubt that Mackie in 1914 when working on kala-azar in Assam actually dissected *P. argentipes* although he was not then aware of its identity In 1922 Swaminath of the kala-azar enquiry in Assam of the Indian Research Fund Association under the direction of Major H E Shortt, I M S, again dissected *P. argentipes* without positive identification In 1922 Major J A Sinton, V C, I M S, in a private communication to Lt-Colonel R Knowles, I M S, pointed out that the known distribution of *P. argentipes* in India corresponded closely with that of kala-azar

From November 1923 to April 1924 the kala-azar enquiry above mentioned dissected numbers of *Phlebotomus* and for the first time some of the flies dissected were definitely identified as *P. argentipes* In all only six identified specimens of female *P. argentipes* were dissected, viz, two on 17th March, 1924, one on 21st March, 1924, two on 28th March, 1924, and one on 3rd April, 1924 All of these were caught in kala-azar houses and all but one contained mammalian blood, the remaining one having an empty gut

In February 1924 Shortt(35) gave what was probably the first published record of *P. argentipes* in Assam in a paper read before the Assam Branch of the British Medical Association In this paper the suspected transmitters of kala-azar were classified into groups as 'probable' and 'possible,' the genus *Phlebotomus* being placed in the former group This paper was read previous to the actual dissection of the first *P. argentipes* on 17th March, 1924, and the finding of mammalian blood in these In December 1924 appeared the most important communication up to that date on the connection between *Phlebotomus* and kala-azar This was the publication of a note by Knowles, Napier and Smith(23) entitled 'On a Herpetomonas found in the Gut of the Sandfly *P. argentipes*, fed on kala-azar patients' These workers showed that the parasites in the peripheral blood of a kala-azar case, in the form of Leishman-Donovan bodies, became converted in the gut of laboratory-bred *P. argentipes* into flagellate forms This important discovery immediately brought to a focus on the genus *Phlebotomus* all the discursive efforts so long vainly expended on a variety of suspected insects The discovery was quickly confirmed by the Kala-azar Commission(8) composed at first of Christophers, Shortt and Barraud, and later of Shortt, Barraud and Craighead, and was extended to include flies caught in nature and fed on kala-azar cases as well as laboratory-bred flies

100 c.c. of serum The quotient, i.e., the globulin/albumin ratio consequently fell from a little less than 3 to the normal 0.66, i.e., 1.15 At this point it remained steady Further the globulin/albumin ratio graph forms a smooth curve indicating that the protein improvement is progressive The rapidity of this change is very striking

#### THE GLOBULIN/ALBUMIN RATIO OF NORMAL SERUM

It is believed that while the absolute amounts of globulin and albumin in the serum may vary, yet the quotient remains constant in a condition of health, and these findings have been used in support of the hypothesis that globulin and albumin are in life combined together, and that protein fractions are artefacts The globulin/albumin ratio is not, however, an absolutely fixed quantity, but varies within narrow limits Moreover, recent immunological work has thrown doubt on this conception of the globulin and albumin existing combined in the serum, as each protein fraction can function separately as an antigen (Hektoen and Welker, 1924) The constancy of the globulin/albumin ratio was first noted by Salvoli, and early observations by Hammarsten gave the figure 0.68 for human serum Other observers have found a somewhat lower quotient Recent observations by Linder, Lundsgaard and van Slyke (1924), who examined sera both by chemical and refractometric methods, give the figure 0.635 We have made most careful observations on this point Our results are shown in Table II above, from which it will be seen that we find the globulin/albumin ratio of normal serum to be 0.662 (1.15)

#### SIGNIFICANCE OF A NORMAL GLOBULIN/ALBUMIN RATIO IN KALA-AZAR

We see that three weeks' specific antimony treatment of a kala-azar case produces a normal globulin/albumin ratio The serum is, however, at this point by no means normal in other respects, for the water precipitation (globulin) test is still positive, and the addition of formalin still produces a definite precipitate which after some time forms a semi-clear gel, and which after 24 hours shows distinct evidence of opacity It is not, however, wholly opaque We have above referred to the extraordinary rapidity with which the ratio becomes normal under treatment It apparently represents the first stage towards normality of the serum, for, as already noted, both the positive formalin reaction and the positive globulin test outlast the point at which the globulin/albumin ratio becomes normal

If, as seems certain, the excess of euglobulin is responsible for the globulin test,—and the formalin reaction is equally certainly associated with the euglobulin then, as the serum proteins are after treatment quantitatively normal, they must be qualitatively different There would seem to be two possibilities here Either a special euglobulin is present, of which we have shown some evidence above, or the total globulin, which is normal in amount, must contain more euglobulin and less pseudoglobulin than a normal serum Possibly both these factors are involved This raises the question of the possible conversion of pseudoglobulin into euglobulin in kala-azar, perhaps into a special euglobulin, and its reconversion during cure This change, if it occurs, will not explain the absolute increase



mouth proper of the fly. It was pointed out that such a fly in attempting to feed could hardly fail to transfer some of its flagellates to the wound made by its bite.

In July 1926 the first specimen of *P. argentipes* caught in nature and showing an infection with *L. donovani* was obtained by the Commission (45).

In September 1926 Shortt, Barraud and Craighead (46) showed that the forms of *L. donovani* found in *P. argentipes* were infective to mice when inoculated intraperitoneally. At this stage in the problem the following data had been ascertained —

- (1) The life-history of *L. donovani* in *P. argentipes* was known
- (2) *P. argentipes* had been found in nature infected with *L. donovani*
- (3) The forms of *L. donovani* found in *P. argentipes* had been shown to be infective to mice
- (4) It was practically certain that *P. argentipes* in biting must inoculate its parasites into the wound made by its bite

The point now seemed to have been reached, in July 1926, when the theory of transmission of kala-azar by the bite of the sandfly would soon be proved to be a fact. So far this hope has been disappointed although a very great amount of experimental work has been done which will be alluded to later.

## VI LIFE-HISTORY OF *Leishmania donovani* (PLATE XXI)

We shall now give a very brief description of the life-history of *L. donovani* in its insect and mammalian hosts as it is essential to an understanding of the *raison d'être* of the transmission experiments to be described.

1 We may commence at the point where *P. argentipes* sucks the blood of a kala-azar case. It takes the aflagellate Leishman-Donovan body contained in a mononuclear or other cell into its midgut.

2 The Leishman-Donovan body may now divide before the appearance of any flagellum, or it may elongate and extrude a short flagellum within a period of two days. Up to this time the newly-flagellated parasites have no power of swimming freely although they may show sluggish undulatory movement.

3 At the end of three days there appear numerous actively swimming flagellates which have developed from those previously showing but sluggish movement. At this stage the infection with flagellates is distributed throughout the midgut of the sandfly.

4 From the fourth day onwards there is an intense multiplication of the forms already present and, at the same time, an invariable tendency towards the concentration of the flagellate infection in the most anterior parts of the midgut. At this stage the fly oviposits and has a second blood meal.

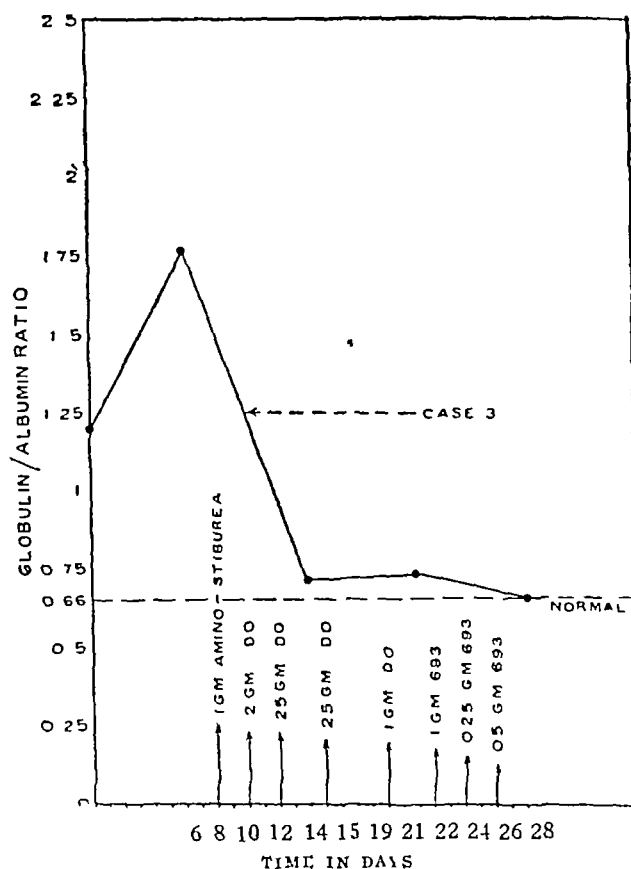
5 By the fifth day the flagellate infection may have reached the pharynx of the fly.

6 By the sixth day the whole pharynx may be invaded.

It seems possible therefore that globulin/albumin ratio estimations may have a useful clinical application, for if subsequent experience should confirm our first impression that a spiky ratio curve is associated with resistance to treatment, it will give the clinician, who may be unaware of the past history of the patient, a clue as to how much treatment to give to such a case

Even if further experience should lead us to the conclusion that a spiky ratio graph is not of special clinical significance, we think it very probable that these graphs, which show in a normally reacting case a sudden fall in the total globulin and a sudden rise in the albumin associated with a smooth globulin/albumin ratio

GRAPH B



curve, may be used as a serological control against which the value of any particular treatment may be judged

### CONCLUSIONS

Our conclusions are therefore as follows —

1 The formol-reacting mechanism in kala-azar is of a double nature. One factor is specific, and is associated with the euglobulin fraction of the serum. The other factor is non-specific, in that the pseudoglobulin plus albumin fraction of a kala-azar serum, a similar fraction from a normal serum, whole normal serum or egg albumin solution may function in this capacity.

The work done in the present series of experiments of the Indian Kala-azar Commission may be summarised in the table given below

Subject of experiment.	Number of flies at supposedly infective stage fed
White mice	3,590
Chinese hamsters	2,325
Human volunteers	1,247
TOTAL	7,162

Some details should here be appended of the precautions taken in the case of the experiments with human volunteers

In the first place the volunteers were obtained from the non-endemic area of Shillong in the Khasi and Jaintia Hills at an elevation of about 5,000 feet. All the men, who were young adults, had lived all their lives in this area. Before commencing the experiments each man submitted to liver puncture, the material obtained being examined by cultural methods. On the day of each separate experiment the volunteer was brought from Shillong to the field laboratory of the Commission at Gauhati by motor. He was then subjected to the bites of *P. argentipes* at the supposedly infective stage, i.e., at least six days after the initial feed on a kala-azar case. Before dusk the volunteer was placed in a perfectly efficient insect-proof room, the only entrance to, or exit from which, was through a double longcloth sleeve attached to a window which could only be reached by a ladder. Food, etc., were provided and the sleeve securely tied and sealed with the official seal. The volunteer was released the following morning and immediately despatched to Shillong by motor. In this way every reasonable precaution against fallacy due to the bites of any insects other than those used in the experiments was taken.

### VIII DIAGNOSIS OF KALA-AZAR

There is not much to be said under this head. Spleen or liver puncture with, when necessary, culture of the material obtained remains the final criterion for the diagnosis of kala-azar. In other words, before one can be absolutely sure that the case is one of kala-azar the parasite in one of its forms must be seen under the microscope.

### IX TREATMENT OF KALA-AZAR

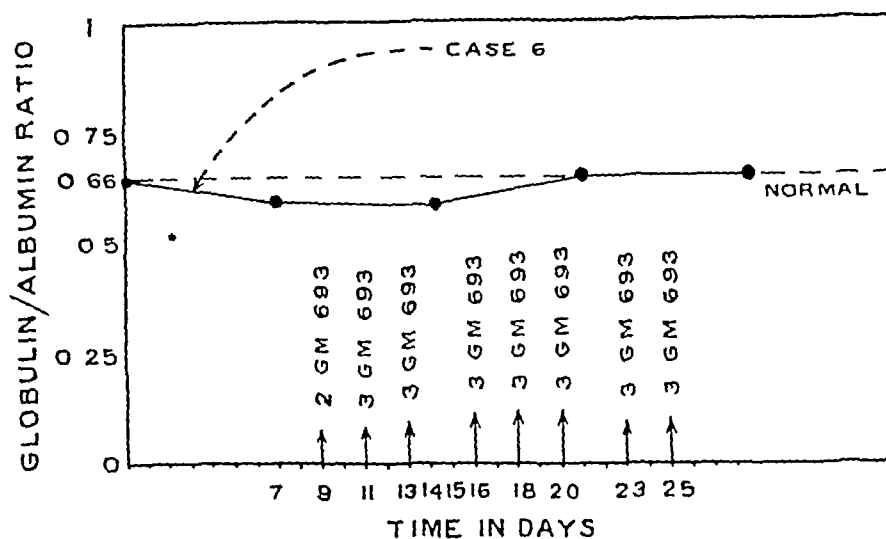
#### *Treatment of Individuals*

Success in the treatment of kala-azar marks one of the greatest therapeutic triumphs of recent times in the realm of tropical medicine. A disease with a mortality in the neighbourhood of 90 per cent, for which, until recently, no

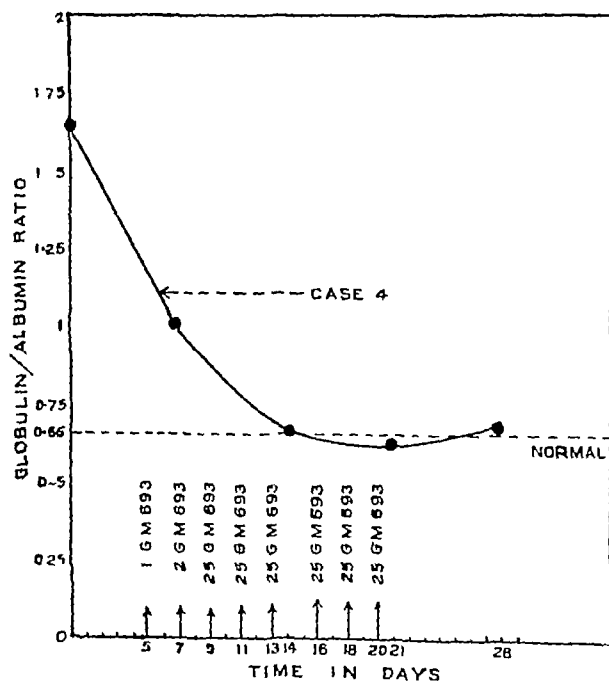
factor may be non-protein in nature, and merely associated with the euglobulin in kala-azar

4 There is considerable increase in kala-azar both of the total serum globulin and of the euglobulin fraction This is accompanied by an absolute

GRAPH D



GRAPH E



Case 4 was a child of 10 years, hence the smaller dosage

decrease of the serum albumin, so that the globulin/albumin ratio which is normally 0.66 becomes greatly raised, in some cases reaching 2.9

*Treatment of cases as a Public Health Measure*

We now come to an aspect of treatment which differs from the treatment of individual cases which we have just been dealing with. Authorities on public health have always, and with justification, looked upon prevention of disease as not only more important but more efficacious than cure. Here, however, they were confronted with a disease the method of transmission of which can still only be guessed at. No adequate preventive measures based on definitely ascertained scientific facts, and applicable as practical measures on a large scale, were known. The decision therefore had to be come to as to whether, in the absence of knowledge of adequate preventive measures, the known weapon of effective treatment could be made use of as a public health measure. The magnitude and cost of such a task must have been apparent and all credit is due to the courage and foresight of McCombie Young(14) who initiated in 1918 and 1919, Taylor(14) who subsequently, during the absence on leave of the former in 1920, further organised and extended the campaign of mass treatment for kala-azar, and Murison(14) who has carried it on and in whose hands it has developed out of all proportion to its more modest beginnings.

The importance and effectiveness of the mass treatment, so unorthodox a weapon in the hands of the preventive medical service, cannot in this case be over-estimated, and the organisation has developed into one of the greatest experiments in public health measures ever adopted. In this connection may be quoted the opinion expressed by Shortt, Das and Chiranjī Lal(47) in a recent publication. Basing their remarks on the fact that an early effect of treatment in most cases of kala-azar is the disappearance of parasites from the peripheral blood, which renders the patient no longer a source for the infection of any transmitting insect, they say —

‘To put it even more strongly we believe, taking into consideration the prevalence of the disease in Assam during the years 1917—1927, that were it not for the widespread sterilisation of the peripheral blood due to treatment, the outbreak in this period would have been more widespread and more disastrous than that of the years 1891 to 1901, when vast areas in Assam became waste land owing to the ravages of the disease. A consideration of the figures showing the numbers treated during the last few years in Assam will show the justification for such an assumption. Thus in the years 1924, 1925 and 1926, 48,770, 60,940 and 46,231 cases respectively were treated. All these cases, even those not completely cured, ceased, for the time at least, to be spreaders of infection, i.e., in the year 1925 alone, there would have been, but for the treatment campaign, 60,940 more sources of infection than there actually were.’

While, therefore, we consider that mass treatment definitely kept the present epidemic within bounds as regards its magnitude and further diffusion, we are less certain that it in any way curtailed its duration. The present recrudescence may be said to have commenced in 1916—1917 and to have reached its height in 1925. It now shows a rapid decline and in another two or three years kala-azar incidence will probably again have reached the comparatively low level of the inter-epidemic periods. If this prediction proves to be true the epidemic, in spite of treatment, will have run, in years, the allotted course which previous

8 By the refractometric method the globulin/albumin ratio can be determined in two hours. A refractometer is not essential, as the estimations may be made by the micro-Kjeldahl method.

9 Strong urea solutions can prevent the formol gel reaction in kala-azar, and can disperse the white gel when formed. This latter action would appear to be a non-specific dispersion of the gel into a sol (peptisation).

10 Urea solutions are in certain cases in conjunction with formalin capable of causing non-specific precipitation. This is being further investigated.

11 The H-ion concentration is higher while the iso-electric point is lower in kala-azar serum than in normal serum. These changes are probably associated with the globulin increase in kala-azar.

The research is being continued. We are further investigating the possibilities of the globulin/albumin ratio graphs. Experiments are also being carried out to determine the relation between kala-azar euglobulin and normal euglobulin.

*Note*—That the above graphs are representative of the serum protein changes occurring in cases of kala-azar under treatment is fully borne out by a much larger series of observations carried out since this paper was completed, and which will be reported in a later publication.

#### ACKNOWLEDGMENTS

We desire to thank Lieutenant-Colonel A. D. Stewart, I.M.S., for very kindly supervising the research during the senior writer's absence on six months' leave. We desire also to thank very specially Dr. L. E. Napier, in-charge, Kala-azar Research Department, School of Tropical Medicine, Calcutta, for his very cordial assistance in this research. The six cases referred to in the text were under his care in the Carmichael Hospital for Tropical Diseases, Calcutta. He has also helped us almost daily in securing a constant supply of suitable sera for analysis, and in supplying much clinical information.

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## EXPLANATION OF PLATE XXI

### *Life-history in the Vertebrate*

- I Small flagellated form of *L. donovani* inoculated by the bite of *P. argentipes*
- II Engulfment of the flagellate by an endothelial or mononuclear cell in which it is conveyed to the spleen, liver, or other tissue
- III *L. donovani* has lost its flagellum and has become an intracellular parasite (Leishman-Donovan body)
- IV Division of *L. donovani* in its intracellular habitat
- V Division into two completed
- VI Multiplication into four parasites
- VII Multiplication into eight parasites
- VIII Cell engorged with parasites
- IX Engorged endothelial cell ruptures
- IX(a) The liberated Leishman-Donovan body taken up by fresh endothelial cells to repeat the process
- X The liberated Leishman-Donovan bodies taken up by mononuclear or polynuclear cells of the circulating blood. In this state they are ingested by *P. argentipes*

### *Life-history in Phlebotomus argentipes*

- (A) The figures in circles denote the number of days which have elapsed since the first feed of the fly on a case of kala-azar
- (B) The lines passing from the circles to the fly denote the parts of the alimentary canal up to which the infection has reached by the corresponding day. The crossing lines for the first four days indicate that the forms present may be found in any situation in the midgut
- (C) The lines radiating from the opposite sides of the circles lead to the forms of *L. donovani* which predominate in the situations indicated on the corresponding days

Lettering —

- B Buccal cavity
- H Hindgut
- M Midgut
- Mp Malpighian tubes
- O Œsophagus
- P Proboscis
- Ph Pharynx
- Pr Proventriculus
- R Rectum

# A BRIEF RÉSUMÉ OF RECENT KALA-AZAR RESEARCH WITH SPECIAL REFERENCE TO INDIA.

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[Received for publication, March 31, 1928]

## SYNOPSIS

- I DISTRIBUTION OF KALA-AZAR IN INDIA
- II THE FORMS OF PREVALENCE OF KALA-AZAR SEEN IN INDIA
  - (a) Endemic Kala-azar
  - (b) Epidemic Kala-azar
  - (c) Dermal Leishmanoid
- III THE PARASITE OF KALA-AZAR
- IV THEORIES OF KALA-AZAR TRANSMISSION.
  - (a) Transmission by direct contaminative means
  - (b) Indirect transmission through an intermediate host
- V THE GENUS *Phlebotomus* AND KALA-AZAR  
*Phlebotomus argentipes* AND KALA-AZAR
- VI THE LIFE-HISTORY OF *Leishmania donovani*
- VII TRANSMISSION EXPERIMENTS WITH *Phlebotomus argentipes*
- VIII DIAGNOSIS OF KALA-AZAR
- IX TREATMENT OF KALA-AZAR
  - (a) Treatment of individuals
  - (b) Treatment as a public health measure
- X THE FUTURE

## I DISTRIBUTION OF KALA-AZAR IN INDIA

THIS is most easily indicated by a map of India (Plate XX) on which the distribution of the disease is marked. A study of this map renders unnecessary any



Between the years 1854—1873 according to Rogers(33), the district of Burdwan in Western Bengal was devastated by an epidemic fever which caused a very high mortality and a considerable decrease in the population as shown by subsequent census returns. Many cases of this fever showed the symptoms now recognised to be typical of kala-azar, especially in its epidemic form, and there seems considerable justification for supposing that much at least of the mortality was due to epidemic kala-azar.

About the same time the district of Dinajpur and Rangpur, adjoining the Garo Hills in Assam, are stated to have been suffering from an epidemic fever similar in character to that in the Burdwan district. Immediately following this outbreak, or possibly even coincident with it, a severe epidemic, now known to have been kala-azar, was found to be raging in the Garo Hills, the nearest point in Assam territory. Attention was called to this outbreak in 1882 when it was already at its height, so it is probable that this district represented the spreading edge of the Rangpur and Dinajpur epidemic, and that the outbreak marked the first mass invasion of the Assam valley by kala-azar. This assumption is favoured by the severity in type of the disease since this appears always to be greatest when virgin territory is attacked.

By 1891 the disease had travelled eastwards and firmly established itself in Nowgong district and all the intervening territory, and from this year until 1901 there raged, especially in the Nowgong area, the terrible epidemic which has made the words 'kala-azar' words of terror in Assam. The appalling effects of this epidemic in the stricken area of Nowgong may be gauged from the fact that in a short period of five years during the height of the epidemic at least one-fourth of the arable land went out of cultivation. Whole villages were wiped out, or deserted by the few survivors, and the land on which they stood reverted to jungle. The Nowgong epidemic may be said to have ended in 1901 but the disease continued to spread slowly in a general easterly direction up the Brahmaputra valley and by 1910 was well established in the Golaghat subdivision. Seven years later the disease had reached the Sibsagar subdivision and about this period may be said to have commenced the epidemic which is now in progress but, at last, rapidly declining. The epidemic, as previous ones, did not merely affect the new territory invaded by the disease but all the territory over which it had passed in its eastward march was similarly affected.

From a consideration of the foregoing brief description of past epidemics, and from our knowledge of the epidemiology of the disease in India amassed by a series of investigators over a considerable period of years, we are able to draw certain generalisations which may prove of value for our future guidance.

One fact which at once emerges is that in India kala-azar is essentially a cyclical disease, i.e., a series of about ten years of epidemic prevalence is succeeded usually by a longer interval of fifteen to twenty years of comparative quiescence, during which the area affected by the epidemic becomes an endemic focus containing a greater or less number of scattered cases not sufficiently numerous materially to affect the vital statistics of the area. The cause of these inter-epidemic quiescent periods is uncertain, but one supposes that in the case of such a fatal disease most of the susceptible individuals have been killed off

of these flies did not take full meals of blood and one sucked up a clear yellow fluid only, presumably serum. Three of these flies were given a second feed on the same case and all the flies were dissected on death. In four flies a heavy infection of flagellates was found, comparable in all respects to the results obtained in flies fed on kala-azar cases. Two out of the four positive flies were among the three which had been fed a second time. This result would seem to imply that the parasite responsible for dermal leishmanoid is probably *Leishmania donovani*, and that its apparent limitation to the skin has not altered its behaviour, as compared with the form found in kala-azar, when it is brought into the environment found in the midgut of *Phlebotomus argentipes*.

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It has been pointed out by McCombie Young(26) that the present epidemic of kala-azar followed in the wake of the influenza pandemic of recent years. It is certainly true that the worst years of the present epidemic were subsequent to the influenza epidemic in Assam in 1919 but there is no doubt that the kala-azar epidemic, as evidenced by an increasing number of cases, had already started on its course by the year 1917, at least two years previous to the advent of epidemic influenza, and that, if the history of previous kala-azar epidemics were to be repeated, the peak of incidence would have been about the years 1922—1925. This actually proved to be the case so that, although the advent of a severe influenza epidemic may have helped to swell the volume of the kala-azar epidemic, there is no definite proof that it either initiated it or materially affected its natural duration.

The question may be asked why the epidemic form of kala-azar is not seen wherever the disease exists. So far as is known, kala-azar, although it has been prevalent in Madras for a long time, has not appeared there in epidemic form. It is possible that the climatic factor may be the determining cause of this since the same phenomenon is seen in the case of another protozoal disease, viz, malaria. Thus the Punjab, where there is a great range of variation in the temperature, both diurnal and seasonal, has, in the past, been subject to very severe epidemic outbreaks of malaria, whereas Madras, where both the diurnal and seasonal range of temperature is comparatively small, has not been subject to such epidemics. In Assam the prevalence of *Phlebotomus* shows very great seasonal variations whereas in Madras these, so far as is known, are much less marked. It is possible that the continuous presence of a transmitting agent, whatever it may be, may result in maintaining continuously a relatively high ratio of immune persons in a population.

#### *Dermal Leishmanoid*

This was the name given by Brahmachari(4) in 1922 to a manifestation of infection with *Leishmania* said not to have been previously described. The lesions of this form of infection are predominantly in the skin and, in their fully developed stage, take the form of small raised papules of red colouration from one to eight millimetres in diameter. Shortt and Brahmachari(39) gave a short account of the histological changes in affected skin and a fuller account has recently been contributed by Acton and Napier(1). The earliest manifestations of this condition are the occurrence of small yellowish depigmented maculae on the skin. These may be distributed over most parts of the body but there is a tendency to the special involvement of the face, ears, upper part of trunk, arms and scrotum. The condition is usually found to have been preceded, at a more or less remote period, by a definite attack of kala-azar which yielded to treatment, but in some instances no such history can be obtained. In connection with the signs of dermal leishmanoid which have just been described the writers are of opinion that the condition was previously described by Christophers(7) in 1904. The following extracts from Christophers' report above referred to show that all the cardinal signs given above were described by him.

expressed that the latter parasite might represent some stage in the life-history of an amphibian or reptilian protozoon

The same opinion was expressed by Shortt (1924) who suggested the probability that the *Crithidia* found in *P. minutus* was the insect phase of *Trypanosoma hemidactyli*

As the result of some recent investigations we have been able to show definitely that the *Crithidia* in question is really a stage in the life-history of a trypanosome of the lizard. Whether this trypanosome is actually the *T. hemidactyli* of Mackie, Gupta and Swaminath is not quite certain since the trypanosomes seen by us in the gecko concerned in our experiments, *Hemidactylus* sp., were morphologically quite distinct from those figured by the above-quoted authors. Those found by them were never seen in the peripheral blood and were only obtained in post-mortem smears from the organs. They were elongate in form. In our specimens the trypanosomes were seen in the peripheral blood during life and were also obtained from the organs and bone-marrow after death and were identical morphologically wherever found. They were broad and stumpy, being roughly oval in shape.

#### EXPERIMENT

Both wild flies caught in nature and laboratory-bred specimens of *P. minutus* were fed upon lizards showing a trypanosome in the peripheral blood. The flies were refed after oviposition, when they survived this act, and, in any case, practically all the flies were dissected at death. A large proportion was found to contain the typical crithidial forms which had previously been found in naturally infected flies. Sections cut of infected flies showed the infection to involve both the midgut and the hindgut but with a tendency to a greater concentration in the latter region the older the infection was. There was no evidence of extension anteriorly towards the mouth parts. The table gives the results obtained.

TABLE

Showing results of feeding *P. minutus* on *Hemidactylus* sp. infected with a Trypanosome

Species of fly fed	No. fed	No. examined after death	No. infected	Percentage infected
<i>P. minutus</i> (bred from eggs)	30	37	22	59.5
<i>P. minutus</i> (caught in nature)	21	18	12	66.7
TOTAL	60	55	34	61.8

The number of flies found naturally infected is not yet known but is, at least, comparatively small. The fallacy which such natural infections might have introduced into the results with flies caught in nature is absent in the case of the bred flies, and the conclusion can, therefore, be drawn that the crithidial

measuring about  $2.1 \mu$  in its longest diameter. Its most evident structural features are a large oval trophonucleus and a rod-shaped parabasal.

In culture or in insects this oval aflagellate body assumes a typical *Leptomonas* form. The body becomes elongated, the trophonucleus and parabasal remain unchanged in form but from the latter there proceeds a long flagellum. These flagellate forms are polymorphic and they have been provisionally classified, on a morphological basis, by Christophers, Shortt and Barraud(10) into six main types.

The chief biological differences between the aflagellate Leishman-Donovan body found in the vertebrate and the flagellate form found in culture or in the insect are two —

(a) The aflagellate form lives continuously at a comparatively high temperature, viz., that of the mammalian host, while the flagellate form can only exist for any length of time at a comparatively low temperature, viz., a temperature not exceeding about  $30^{\circ}\text{C}$ .

(b) The aflagellate form occupies an intracellular habitat, while the flagellate form is free-swimming.

#### IV THEORIES OF KALA-AZAR TRANSMISSION

Since the discovery of the parasite of kala-azar theories of transmission have been many and varied, but so far they have all remained mere theories. The various suggestions put forward may be said to have crystallised into two general hypotheses —

- (1) That transmission was direct by contaminative means
- (2) That transmission was indirect through the intervention of an intermediate host

The theory of direct contaminative transmission appeared to be supported by the unhygienic conditions which are usually associated with the presence of kala-azar. According to this theory the parasite of kala-azar must leave the body in some of the bodily secretions or excretions and so be directly conveyed to other individuals. The discovery by Shortt(34) that the parasite is not infrequently found in the urine lent some support to this view, but our knowledge of the bionomics of the parasite appear to render it improbable that infection can be contracted by this means.

Indirect transmission through an intermediate host has, on the analogy of some other protozoal diseases, been the most favoured hypothesis. Many insects have been suggested as possible carriers of infection, those most favoured being the bed-bug *Cimex hemiptera*, *Triatoma rubrofasciata* and sandflies of the genus *Phlebotomus*.

Most of the suspected insects have now been ruled out as the result of experimental investigation of their claims as transmitters, the only survivors being the genus *Phlebotomus*. This genus has now assumed such importance in connection with the problem of transmission of kala-azar that it is necessary to treat it at some length.



studied and we feel convinced that only further study is required to determine the trypanosomes of which many of the known species of *Crithidia* are the arthropod phase, in any case no hard and fast rule can any longer be drawn between the two genera

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In March 1925 Christophers, Shortt and Barraud(9) gave the first description of the flagellates encountered in infected flies and of their location in the gut of the insect. They also described for the first time the invasion of the pharynx of the fly by flagellates. At the same time they investigated the possibility of infecting the adult sandflies through the larval and pupal stages by feeding the larvæ on old cultures of *L. donovani* but without result(12).

In April 1925 the same workers(11) proved by a considered series of controls with laboratory-bred flies that the flagellates seen in *P. argentipes* after feeding on kala-azar cases really were *L. donovani*. In July 1925 P. J. Barraud, F. E. S. F. Z. S.,(3) of the Kala-azar Commission made a sandfly survey of Madras City and established the presence in that city of four species of *Phlebotomus*.

Up to this time the great difficulty experienced in all experiments devised to prove the transmission of kala-azar by the bite of the sandfly lay in the fact that the fed flies almost invariably died by the fifth day after the initial feed and before a second feed could be taken. The cause of death was invariably the failure of the fly to oviposit, without which act a second blood meal was not possible. In September 1925 the Commission overcame this difficulty by the discovery of a technique(41) the use of which enabled the flies to oviposit and take many successive meals, oviposition occurring between each two meals. This technique opened up the way to an extensive series of transmission experiments by the Commission and other workers.

In November 1925 as a direct result of the application of this technique Shortt, Barraud and Craighead(40) announced the discovery of massive infections of the pharynx and infections of the buccal cavity in *P. argentipes* which had received two or three blood meals. These infections were demonstrated in microscopical sections of infected flies. By December 1925 the same workers(42) had elucidated for the first time the complete life-history of *L. donovani* in *P. argentipes*, and this was subsequently published in April 1926. In February 1926 there was published the 'Kala-azar Memoir' which included most of the work done by the Commission up to that date as well as some papers by other workers. Besides the work already alluded to, the memoir contained a description of the anatomy of the head of *P. argentipes*(13) which, although published under three names, was entirely the work of Christophers. Among included papers not the work of the Commission was one by Napier and Smith(30) on the bionomics of *P. argentipes* with special reference to conditions in Calcutta and one by Napier(29) entitled 'Epidemiological Consideration of the Transmission of Kala-azar'. In the latter paper twenty-one conditions are laid down as favourable for the hypothetical transmitter of kala-azar (insect or otherwise) but many of these will not stand the test of universal application even in India alone. The claims of various helminths and arthropods as possible transmitters of kala-azar are also considered in the light of known epidemiological facts. In May 1926 a technique(43) was elaborated by the Commission for the breeding, throughout the entire year, of *P. argentipes* on a large scale from specimens themselves artificially reared. In June 1926 Shortt, Barraud and Craighead(44) described the occurrence of a massive infection of the buccal cavity of *P. argentipes* with *L. donovani* in which the most anteriorly placed flagellates protruded from the

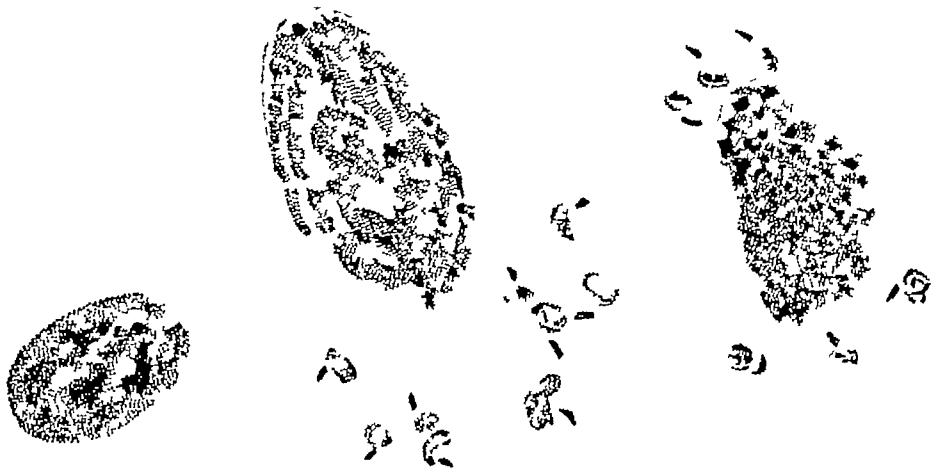
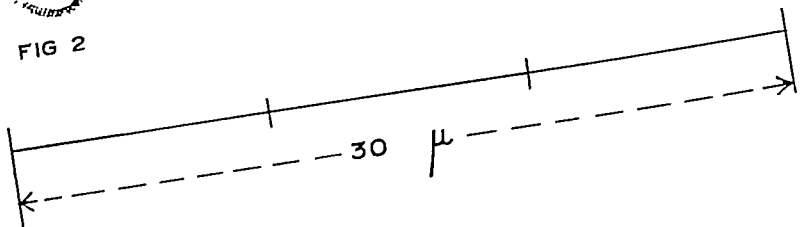


FIG 1



FIG 2

FIG 3



7 By the seventh day the infection may reach the junction of the pharynx and buccal cavity

8 By the eighth or ninth day the buccal cavity may show a massive invasion and the fly is ready to transmit its infection The most anteriorly placed flagellates are usually small elongated forms with well developed flagellum

9 If the fly now bites a mammal the natural assumption is that some of the anteriorly-placed flagellates will be ejected into the wound either singly or as a small group

10 The flagellate in the wound is now engulfed in a phagocytic endothelial cell and loses its flagellum in the process The parasite thus acquires an intracellular habitat

11 The body of the parasite contracts and it assumes the typical Leishman-Donovan body form The time occupied between the entry of the free flagellate form into the wound and its development into an intracellular Leishman-Donovan body may vary, but in one observed case the transformation was completed at the end of  $3\frac{1}{2}$  hours, when cultural forms of the parasite were used

12 The parasitised endothelial cell now gains the circulation directly, or through the lymph channels, and is carried to such an organ as the spleen or liver where it is arrested

13 The parasite proceeds to divide inside its host cell by a series of binary fissions until the cell is fully distended and finally ruptures setting free its load of parasites

14 Some of these are taken up by fresh phagocytic cells of the organ in which they have been liberated while others may be taken up by endothelial or mononuclear cells of the capillaries and so enter the general circulation where they are met with in the peripheral blood, thus completing the life-cycle of the parasite

## VII TRANSMISSION EXPERIMENTS WITH *Phlebotomus argentipes*.

We do not wish at present to go into great detail with regard to these experiments, which will be more fully described elsewhere, as they are not yet completed and no final result has been obtained A summary only will therefore be given in order to show the extent of the experiments and the precautions taken to prevent fallacy The experiments have been carried out with white mice, Chinese hamsters (*Cricetulus griseus*) and human volunteers In the details to be given below any results of experiments already published are not included so that they do not represent the full volume of transmission experiments with *P. argentipes* carried out by the Kala-azar Commission It should be pointed out that similar experiments, although on a smaller scale, have been carried out by the Ancillary Kala-azar Enquiry at Calcutta but, as no results have been published, we cannot include these in the present review Similarly the workers in China, especially Young and Hertig(51) have carried out a large number of transmission experiments, although with different species of sandfly, and the Kala-azar Commission of the Royal Society, Hindle and Patton (20), have done somewhat parallel work



effective treatment was known, has been converted into a disease with a recovery rate of at least 90 per cent in properly treated cases of all kinds while, if cases are recognised and treated in their early stages, we may now say that practically every case can be cured. The apparent miracle has been brought about in two stages

The first stage is represented by the introduction by Vianna, in 1913(50) and Di Cristina and Caronia in 1915(18) of the use of antimony salts in the treatment of *Leishmania* infections. In 1915 Rogers(32) introduced the treatment into India for kala-azar and it was quickly found to be most effective. In 1917 Knowles,(22) working in Shillong, made a valuable contribution to the therapeutics of kala-azar by standardising the dosage and methods of administration of antimony salts, and his work has been a basis of comparison for subsequent research on the drug treatment of kala-azar. The treatment was found to be most effective both with potassium and sodium antimony tartrate, but the time required to produce cures was prolonged, involving a course of about forty intravenous inoculations within a period of two to five months.

The second stage in the development of the present highly efficient treatment was the introduction of organic compounds of antimony. In 1922 U N Brahmachari,(5) working under the auspices of the Indian Research Fund Association, commenced to prepare and experiment with various new antimony compounds. At the request of Lieutenant-Colonel E D W Greig, Director of Medical Research, Brahmachari supplied samples of some of his products to one of us for trial at the Special Kala-azar Hospital, Shillong. One of these products was urea stibamine. Of this drug, Brahmachari, who had tried it on eight cases, said 'Urea stibamine has been found useful in the treatment of kala-azar'.

As soon as the drug was tried in Shillong by Shortt and Sen(36) the results in the first few cases showed such an astonishing advance on those obtained with the older preparations that a larger supply was called for and supplied by Brahmachari and further experimentation carried out. This confirmed the good results(37, 38) at first obtained and, after certain difficulties in manufacture had been overcome, the drug established itself, and still remains, as a great advance on the older antimony salts and as good a therapeutic agent in the treatment of kala-azar as any of the later organic preparations of antimony subsequently put on the market.

After the introduction of urea stibamine various other organic antimony preparations came on the market, the first of these to be placed in the same class as urea stibamine for effectiveness being Von Heyden 471 which was experimented with by Napier(27) and stibamine glucoside (now called under the trade name Neostam) which has been favourably reported on by Hodgson(21), Greig and Kundu(19) and Napier(28).

The great advance which all these preparations mark in the treatment of kala-azar is shown by the fact that the treatment, which previously lasted for two to five months, now lasts the same number of weeks. In addition, many of the cases which proved refractory to the older antimony salts yielded to treatment with the newer preparations.

Evidence of viability of street virus in infected brains was found after 72, and possibly 84 hours, exposure (Cunningham, Nicholas, and Lahiri, 1927)

The results for infected cords are set forth in the two accompanying tables. Arguing from the resistance of fixed virus in cords which had previously been determined (Cunningham, Nicholas, and Lahiri, 1926) preliminary exposures of 6 and 7 hours were made. It was ultimately found, however, that there was little difference in the resistance of street virus, whether situated in cord or brain, and that exposures of 96 hours to the fluid, and no less than 144 hours to the vapour, were required to kill the virus. These periods are somewhat longer than those found necessary in our experiments with brains but they may be due to the fact that different and possibly more resistant strains of virus were used in the latter series of experiments.

One important fact stands out, however, from this comparison. The viability of the virus under such conditions does not depend solely upon the thickness of nerve tissue to be penetrated, but is obviously due in great part to the resistance of the virus itself, as the amount of nerve tissue is much less in the case of the cord than of the brain and yet the viability of the virus in both situations has been found to be very similar.

### CONCLUSIONS

1 The resistance to ether of street virus in infected cords has been tested both by immersion in the fluid and exposure to the vapour.

2 Living virus has been found in cords immersed in ether fluid for periods up to 84 hours but not 96 hours.

3 This period is extended to 120 hours and 144 hours in the case of exposure to the vapour alone.

4 The virus in cords is as resistant to the action of ether as the virus situated in brain tissue. This observation indicates that the resistance to the action of ether is an innate property of the virus itself and not due merely to the rate of penetration of the ether into nerve tissue.

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epidemics have led us to expect, i.e., a period of 10 or 12 years, although treatment will have prevented its attaining a disastrous magnitude and the conquest of fresh virgin territory to be converted into an endemic area

## X THE FUTURE

We have shown how important, economically, years of epidemic prevalence of kala-azar are to the province of Assam. Any question of economic importance becomes automatically one of political importance and hence, during the last nine or ten years, kala-azar has necessarily loomed large in the political sphere in Assam.

The consequence of this has been that the Government of Assam, with the approval of successive ministers as well as of the general public, has found little or no opposition to the expenditure of larger and larger sums of money on measures of treatment and, *ipso facto*, prevention of kala-azar, since the beneficial effects of these measures are too patent to escape the observation and appreciation even of so conservative a people as the Assamese, in general, are. The very success of these measures, however, and the decline in the epidemic are a source of danger for the future.

Present indications show that in another year or two the disease in Assam will have regained the character prevalent in the inter-epidemic periods when it exerts no undue influence on the mortality and health returns of the province as a whole. It will then cease to have any political importance and no kudos will be gained by those who make special efforts to extinguish the few remaining embers of infection left behind as the result of the present epidemic. It cannot, then, be too strongly impressed on the governments concerned that this very time, when the numbers of cases are comparatively few, is the time 'par excellence' to prosecute with the greatest vigour measures of treatment and prevention with the view of eradicating from the country all sources of future danger represented by the relatively few cases continually occurring in the endemic areas.

No predictions can safely be made as to the possible occurrence of future epidemics. The present outbreak is the first in which any effective treatment has been available for the disease and what effect the almost universal application of this will have on the occurrence and date of any future epidemic it is impossible to say. We can only repeat, and ask others to repeat, that the measures taken, by treatment and otherwise, within the next few years are likely to be more important, and to have a more far-reaching effect, if resolutely pursued, than the most intensive anti-kala-azar measures carried on during the height of the epidemic years.

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TABLE I—(contd)

ETHERISED COBDS.		INOCULATION						SUB PASSAGE						
No of hours immer- sed	No of cords treated	Sample taken from	No of rabbits inocu- lated	Deaths	No of days to		Negri bodies	Cerebro spinal fluid	REMARKS	No of rabbits sub pas- saged	No of days to		Negri- bodies	REMARKS
					First sym- ptoms	Death					First sym- ptoms	Death		
24	1	Outside Inside Mixture	1 1 1	1 1 1	13 16	14 17 4	++	St St	Rabies Rabies Diarrhoea, no sub passage				-	
48	1	Outside Inside Mixture	1 1 1	1	37	39	+	St	Alive after 166 days Alive after 165 days Rabies					
72	1	Outside Inside Mixture	1 1 1	1 1 1		17 21 24	- ++	St St St	Rabies Rabies	1				Alive after 187 days
84	1	Outside Inside Mixture	1 1 1	1	43	47	+	St	Alive after 162 days " " Rabies					
96	1	Outside Inside Mixture	1 1 1	1		124	-	Con	Alive after 150 days " " Not rabies					
120	1	Outside Inside Mixture	1 1 1						Alive after 149 days " " " "					
St =Sterile Con =Contaminated														

St =Sterile

Con =Contaminated

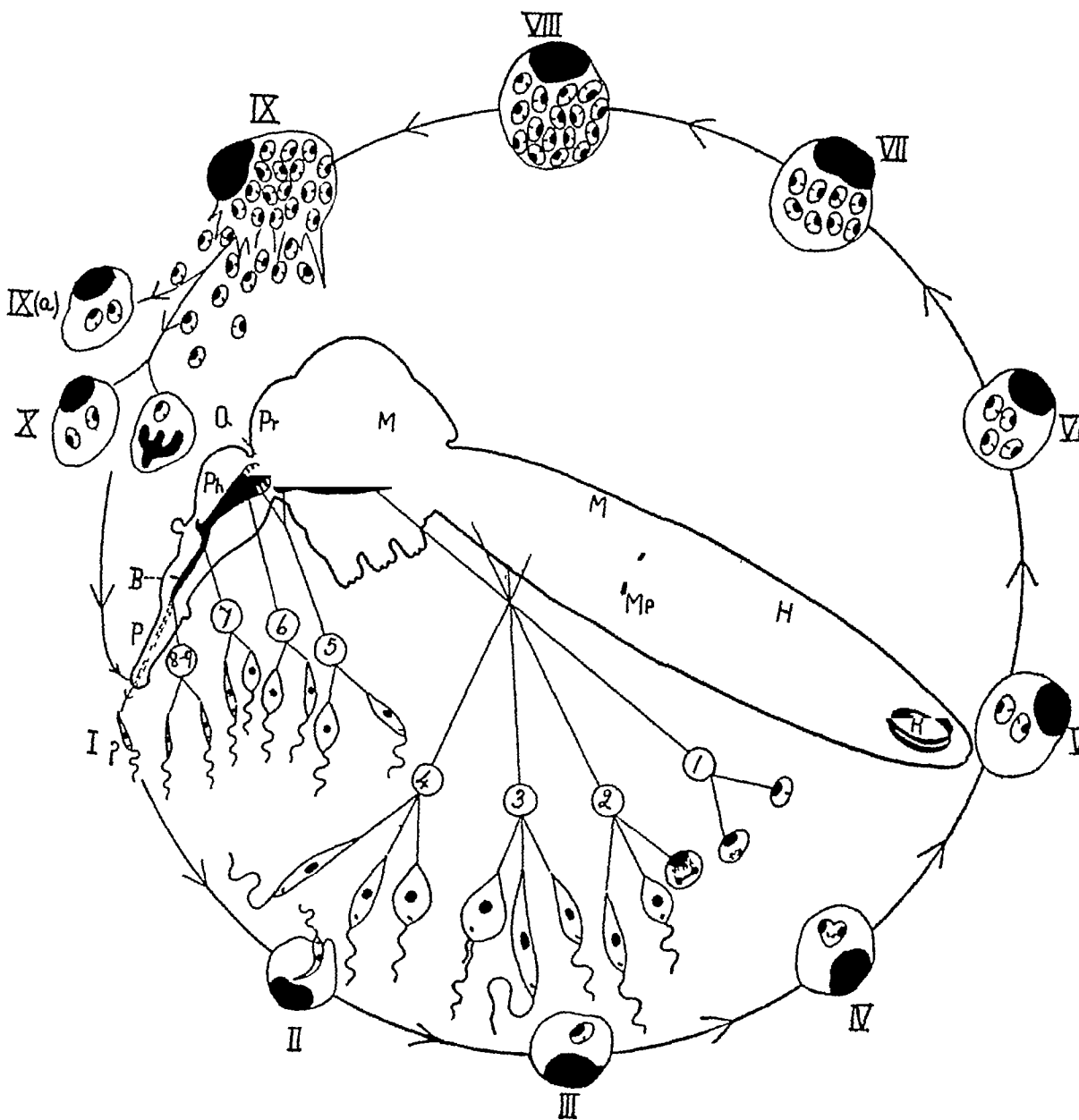
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TABLE II—(contd.)

TABLE II—(contd.)

ETHERISED CORDS		INOCULATION							REMARKS	SUB-PASSAGE			REMARKS
No of hours exposed	No of cords examined	Sample taken from	No of rabbits inoculated	Deaths	No of days to		Cerebro spinal fluid	Negri bodies		No of rabbits sub-passaged.	First symptoms	Death	
					First symp toms	Death							
12	1	Outside Inside Mixture	1 1 1	1 1	11	14 1 6	St St	+ —	1			Alive 8 months later	
14	1	Outside Inside Mixture	1 1 1	1 1 1	12 13 12	19 14 15	St St St	+ + +					
16	1	Outside Inside Mixture	1 1 1	1 1	(Killed) 13 13	206 16 16	St St St	— + +					
24	1	Outside Inside Mixture	1 1 1	1 1 1	13 13 14	17 16 17	St St St	+ + +					
48	1	Outside Inside Mixture	1 1 1	1 1 1	15 15 18	17 20 20	St St St	+ + +					
72	1	Outside Inside Mixture	1 1 1	1 1 1	14 13 14	18 16 16	St St St	+ + +					

PLATE XXI



PROVISIONAL LIFE-HISTORY OF *L. DONOVANI*



## NOTE ON DERMAL LEISHMANOID

BY

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[Received for publication, March 31, 1928]

SINCE the first description of this condition by Brahmachari (1922) many cases have come to light, especially in Calcutta, and the condition seems to be by no means uncommon in endemic areas

It has usually been concluded that the parasite causing the condition was actually *Leishmania donovani* since a majority of the cases gave a definite previous history of kala-azar

In order to help to settle this question use was made of a case recently seen by us in Assam, by feeding *Phlebotomus argentipes* on the lesions. If the parasites ingested by the flies behaved in a manner exactly similar to those whose source was a definite case of kala-azar it would be strong presumptive evidence of the identity of the parasites in the two conditions

The history of the case in question was as follows —Bikhu, male, age 20, had 30 intravenous injections of sodium antimony tartrate a year previously for kala-azar, with apparent cure. The nodules appeared on the face, arms and trunk some time after treatment had been stopped, but he could not say definitely how long after. The condition had become progressively worse and showed no tendency towards cure. Smears from an excised nodule showed fairly numerous *Leishmania*. Microscopical examination of the peripheral blood was negative

### EXPERIMENT

Certain of the lesions on the face were of considerable extent, even up to about 1 cm in diameter. On these lesions specimens of *Phlebotomus argentipes* were fed in test tubes on nine occasions, a total of 25 flies being fed. A few

guinea-pigs, 53 hours immersion in ether was required to kill the virus in the superficial parts and 120 hours in the case of virus situated in the deeper layers of the brain Alivisatos (1922), experimenting with dogs and sheep, showed that, with his virus fixé, viability was uncertain in brains exposed for 120 hours and death was the rule after 144 hours immersion

In our hands the Paris virus fixé in infected brains has withstood the action of ether in both the outer and inner layers of the brain for 144 hours but has succumbed after an exposure of 168 hours In 'cords' the results have been somewhat irregular but evidence of viability has been obtained in samples from the inner layers after immersion for 96 hours According to our experiments, therefore, the resistance of the 'Paris' virus fixé to ether is rather greater than that claimed for it by Remlinger and Alivisatos (if the fixed virus used by the latter came originally from the same source)

There is now no question that the 'Paris' strain is much more resistant than the fixed virus in use in Kasauli and in other institutes in India, and that it is even more resistant than the various strains of Indian street virus tested under similar conditions by us (Cunningham, Nicholas and Lahiri, 1927, 1928)

It is of interest to note in this connection that the length of time required to produce symptoms and death in the sub-passage rabbits differs considerably in the 'Kasauli' and 'Paris' viruses

With Kasauli fixed virus the rabbits generally show symptoms on the 7th and die on the 9th day, whereas with the Paris virus fixé symptoms do not appear until the 9th day while death is delayed until the 11th or 12th day Our experience with the Paris virus coincides with that of the authorities of the Pasteur Institute in Paris It would appear, therefore, that the Paris virus, although much older than the Kasauli virus, is a much harder virus and has retained the characteristics of the virus of the street to a far greater extent than has the Kasauli fixed virus

#### SUMMARY AND CONCLUSIONS

1 The resistance to ether of the virus fixé in use in the Pasteur Institute in Paris has been tested both in infected brains and cords

2 Living virus has been found in infected brains after 144 hours immersion but not after 168 hours In infected cords the virus was found alive in the inner parts of the cord after 96 hours immersion

3 Our results give rather higher values than those recorded by either Remlinger or Alivisatos

4 The 'Paris' fixed virus is far more resistant to the action of ether than the Kasauli fixed virus It is also more resistant than any of the strains of Indian street virus so far tested

#### ACKNOWLEDGMENTS

Our best thanks are due to M Calmette, Director of the Pasteur Institute, Paris, who kindly supplied us with a sample of the historic virus fixé of that Institute and to Lieutenant-Colonel F P Mackie, I M S, for bringing it to India for us

# PRELIMINARY NOTE ON THREE SPECIES OF *TRYPANOSOMIDÆ*.

BY

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AND

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(From the Kala-Azar Commission)

[Received for publication, March 31, 1928]

## *LEISHMANIA HEMIDACTYLI* (*HERPETOMONAS HEMIDACTYLI*)

THIS parasite was first described under the name of *Herpetomonas hemidactyli*, by Mackie, Gupta and Swaminath (1923) who obtained the cultural forms from a common wall gecko *Hemidactylus* sp. in Assam. This name was emended by Wenyon (1926) who placed the parasite in the genus *Leishmania* on the ground that part of the life-history was passed in a vertebrate host.

The justification for this change is now evidenced by the finding by us of the typical Leishman-Donovan body form of the parasite in the gecko.

A gecko (*Hemidactylus* sp.), examined on 5th July 1927, showed in the peripheral blood typical aflagellate *Leishmania* elements. The bodies were found on two occasions in this lizard and were either contained in a mononuclear cell or were lying beside one and had evidently been displaced when the cell was damaged in making the smear.

The individual parasites measured about  $2.5\mu$  in their greatest diameter and contained the typical large trophonucleus and rod-shaped parabasal (Plate XXII, fig. 1). The lizard was sacrificed, and cultures made on NNN medium from the liver gave an abundant growth of typical *Leptomonas* forms which has been maintained in culture without difficulty. Examination of the cloaca and intestine of the lizard revealed no flagellate infection.

## *TRYPANOSOMA* (?) *HEMIDACTYLI* (PLATE XXII, FIG. 2)

Mackie, Gupta and Swaminath (1923) described a trypanosome found in the internal organs of *Hemidactylus* sp. in Assam, and in the same publication recorded the finding of a *Critidia* in *Phlebotomus minutus*. The opinion was



TABLE II  
Showing the effect of immersing cords infected with the *Virus Fixé* of the Pasteur Institute, Paris, in ether for periods varying from 72 to 144 hours

ETHERISED CORDS		INOCULATION					REMARKS	Cerebro spinal fluid	SUB PASSAGE			Cerebro spinal fluid	REMARKS
No of hours immer sed	No of cords treated	Sample taken from	No of rabbits inocu- lated	Deaths	No of days to				No of rabbits sub-pas- saged	First symp- toms	Death		
72	1	Outside	1	1	10	13	Alive after 180 days Not Rabies	1	8	11	St	Rabies	
		Inside	1	1		9							
		Mixture	1	1									
84	1	Outside	1	1	10	13	Rabies ?	1	9	3	St	Not rabies Rabies	
		Inside	1	1	19	20		1		10			
		Mixture	1	1									
96	1	Outside	1	1	11	14	Alive after 157 days Alive after 157 days	1	7	10	St	Rabies	
		Inside	1										
		Mixture	1										
120	1	Outside	1	1	32	33	Alive after 157 days Alive after 157 days	1				Alive after 145 days	
		Inside	1										
		Mixture	1										
144	1	Outside	1			32	Alive after 166 days Alive after 166 days	1		1	Con	Not rabies	
		Inside	1										
		Mixture	1										

St = Sterile      Con = Contaminated.

infection seen in *P. minutus* in Assam is actually a phase in the life-cycle of a trypanosome of the lizard *Hemidactylus* sp

TRYPANOSOME CONORHINI (*CRITHIDIA CONORHINI*  
DONOVAN, 1909)

The great resemblance shown by this parasite of *Triatoma rubrofasciata* to the forms of *Trypanosoma cruzi* in *Triatoma megista* would naturally have led one to suppose that it also was a phase in the life-cycle of a trypanosome

Lafont (1912) in Mauritius has shown that the parasite of *T. rubrofasciata* in that island is inoculable to certain laboratory animals and appears in their blood in the form of a trypanosome. The infection is said to be apparent in the peripheral blood after the lapse of thirty hours and to persist for five days only

In order to confirm these findings for the parasite of *T. rubrofasciata* in India we have performed certain preliminary experiments

EXPERIMENT

On 14th February 1928, two white mice and one guinea-pig were inoculated intraperitoneally with the gut contents of eight *T. rubrofasciata* (late nymphal and adult stages) collected in Assam. These animals were examined at frequent intervals

On 24th February 1928, the guinea-pig was found dead and autopsied with negative findings

On 27th February 1928, both the mice showed a trypanosome infection of the peripheral blood (Plate XXII, fig 3). This infection was present for two days subsequently and after this period has not again been noted. We think it probable that the infection may have been present at an earlier date than that on which we detected it as the slight infections are very difficult to demonstrate by direct examination

As the mice used belonged to a stock with which we have been working for five years, and which have never shown any blood parasites, the presence of a natural infection with trypanosomes may be ruled out. The only alternative is that the parasites in the mice represented the vertebrate phase in the life-cycle of a trypanosome while those in the bug represented the insect phase

*T. rubrofasciata* in Assam is comparatively common in village houses and the immature forms of all stages are most commonly met with in the nests and boxes of the pigeons which many villagers keep. The possibility, therefore, that the trypanosome may be that which has been reported from pigeons should be borne in mind. On the other hand, the close resemblance of *Trypanosoma vespertilionis* to *T. cruzi* shown by Chatton and Courrier (1921) indicates the possibility that a bat may be the vertebrate host

Further work on these three parasites is in progress

In 1924 one of us expressed the opinion that the genus *Crithidia* might, as our knowledge of it increased, have to be sunk as being synonymous with the genus *Trypanosoma*. This opinion is being strengthened the more the genus is





technique was similar in every respect to that employed in previous experiments. The results are given in the table.

Definite evidence of viability was found in the 'inside' sample immersed for 84 hours. The result of the inoculations with the inner sample exposed for 96 hours is also suspicious. The 'sub-passage' from this rabbit unfortunately died early from another cause. Positive proof of the disease is, therefore, wanting. The death of the '120 hour' rabbit was probably not due to rabies because the sub-passage rabbit remained alive. The above results show that this particular fixed virus does not correspond either to the Kasauli fixed virus No 1 or to the Paris fixed virus. It approaches Kasauli fixed virus No 1 in the time required to produce symptoms and death in the passage animals but it is more like the Paris fixed virus and the other strains of Indian street virus tested with regard to its resistance to ether.

It would appear from the whole series of experiments, carried out on this subject, that different strains of rabies virus, both street and fixed, vary considerably in this respect, and that no definite periods for attenuation and death can be laid down which will be generally applicable to all strains of virus. It follows, therefore, that each strain to be used for the etherised method of anti-rabic inoculation must be carefully tested before use so as to determine the times at which attenuation and death of the virus occur.

#### SUMMARY AND CONCLUSIONS

1 A freshly fixed strain of rabies virus (K F V No 2) has been tested with regard to its resistance to ether.

2 With regard to 'Incubation Period' and 'time from inoculation to death' the virus conforms closely to Kasauli fixed virus No 1 (the original Kasauli fixed virus).

3 In infected brains this virus remains alive in the samples taken from the inner layers of the brain after immersion for 84 and perhaps 96 hours.

4 In this respect, therefore, this particular strain does not conform to the results previously obtained with either the Kasauli fixed virus No 1 or the fixed virus of the Pasteur Institute, Paris, but is more akin to the strains of Indian street virus tested.

5 When the whole series of experiments carried out on this subject is reviewed it is obvious that different strains of rabies virus, both fixed and street, vary considerably in regard to their resistance to ether and that no definite period can be laid down which will be universally applicable. Each strain of virus must, therefore, be carefully tested before use.

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EXPLANATION OF PLATE XXII

- Fig 1 *Leishmania hemidactyli* in peripheral blood of the gecko *Hemidactylus*  
sp  
„ 2 *Trypanosoma* (?) *hemidactyli* seen in the blood of gecko *Hemidactylus*  
sp  
„ 3 *Trypanosoma conoium* in the blood of white mouse

**TABLE**  
*Showing the effect of immersing a freshly Fixed Rabies Virus (K F V No 2) in ether for periods varying from 24 to 144 hours*

FRESHLY BRAINS		INOCULATION						REMARKS	Cerebro spinal fluid	SUB PASSAGE			Cerebro spinal fluid.	REMARKS
No of hours immer sed	No of brains tested.	Sample taken from	No of rabbits inocu lated	Deaths	No of days to		No of rabbits sub pas saged			First symp- toms	Death			
					First symp toms	Death								
24	1	Outside Inside Mixture	1 1 1	1 1 1	9 9 7	12 11 9	1 1 1	6 7 7	10 10 11		St St St	Rabies Rabies Rabies		
48	1	Outside Inside Mixture	1 1 1	1 1 1	9 8 8	11 11 10	1 1 1	6 6 7	9 9 10		St St St	Rabies Rabies Rabies		
72	1	Outside Inside Mixture	1 1 1	1 1 1	10 10 10	11 12	1 1	6 6	8 10		St St	Rabies Rabies		
84	1	Outside Inside Mixture	1 1 1	1	10	12	1	6	8		St	Rabies		
96	1	Outside Inside Mixture	1 1 1	1		10	1		3			Diarrhoea not rabies		
120	1	Outside Inside Mixture	1 1 1			10						Alive after 74 days		
144	1	Outside Inside Mixture	1 1 1											

St = Sterile

# AN INVESTIGATION INTO THE VALUE OF AN ETHERISED VACCINE IN THE PROPHYLACTIC TREATMENT OF RABIES

## Part III.

### THE ACTION OF ETHER ON STREET VIRUS IN INFECTED CORDS

BY

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In our last paper we described the effect of ether on street virus as found in brain tissue. In the present note we propose to deal with the resistance of street virus in infected cords. Our technique remains the same as that described in our previous papers with the exception that we have used half cords for each exposure, thus enabling us to give a parallel series for exposure to the fluid and the vapour. Six different strains of virus have been used for the series. The details of these are given in the following Table —

Virus	Sub passage in rabbit	DAYS TO	
		First Symptoms	Death
1	3	15	15
2	2 to 4	8 to 13	12 to 20
3	4	12	15
4	4	12	15 to 17
5	2	12 to 16	22
6	2	10	15



TABLE II  
*Maximum number of Deaths from Smallpox in the period 1896 to 1922*

Provinces	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
M <sub>1</sub>	3,795	3,330	3,118	2,205	2,509	1,670	1,216	1,115	824	770	1,084	2,590
M <sub>2</sub>	1,700	2,080	2,773	2,702	2,291	1,992	1,891	1,923	1,504	1,380	1,483	1,373
M <sub>3</sub>	1,423	1,515	1,933	1,628	1,325	1,246	1,151	1,077	1,054	928	973	1,290
B <sub>V</sub>	2,254	3,828	3,883	3,023	1,532	643	418	250	171	287	381	1,010
P <sub>F</sub>	8,865	8,786	7,037	5,967	8,047	6,581	4,192	2,081	1,040	1,448	3,656	7,902
U P	6,529	7,076	12,605	21,313	19,224	10,723	5,856	2,794	1,206	785	2,011	4,296
C. P	1,035	1,314	1,477	1,574	1,496	1,298	966	614	397	373	301	659
B & O	2,319	2,227	4,102	5,229	4,722	4,673	2,332	1,174	746	638	679	1,827
A	675	758	1,210	1,130	1,088	752	445	314	266	236	301	463
B <sub>1</sub>	1,003	1,035	2,357	3,602	4,343	4,094	2,448	1,241	709	349	357	709
B <sub>2</sub>	1,448	1,984	2,855	2,307	1,473	737	383	278	133	116	215	585
B <sub>3</sub>	1,528	2,010	2,567	1,722	1,527	1,388	763	367	398	303	329	1,048
B <sub>4</sub>	1,802	2,802	4,413	3,788	2,612	1,796	963	591	544	442	403	1,359

TABLE I

Showing the effect of immersing 'Street Virus' Cords in ether for periods varying from 7 to 120 hours.

Showing the effect of immersing Sterilized Cords in Immersion Fluid

ETHERISED CORDS			INOCCULATION						REMARKS	SUB PASSAGE				REMARKS	
No of hours immersed	No of cords treated	Sample taken from	No of rabbits inoculated	Deaths	No of days to		Cerebro spinal fluid	Negri bodies		No of rabbits sub pas saged	No of days to		Cerebro spinal fluid		Negri bodies
					First symp toms	Death					First symp toms	Death			
7	1	Outside Inside Mixture	1 1 1	1 1 1	14 13	15 16	St St	++	Alive after 177 days Rabies Rabies						
8	1	Outside Inside Mixture	1 1 1	1 1 1	13 12 10	19 17 10	St St St	+++	Rabies Rabies Rabies						
9	1	Outside Inside Mixture	1 1 1	1 1 1	9 10 16	10 6 16	St St St	++-	Rabies Rabies Rabies	1	14	16	St	+ Rabies	
12	1	Outside Inside Mixture	1 1 1	1 1 1	12 12 17	16 17 20	St St St	+++	Rabies Rabies Rabies						
14	1	Outside Inside Mixture	1 1 1	1 1 1	12 12 13	17 17 15	St St St	+++	Rabies Rabies Rabies						
16	1	Outside Inside Mixture	1 1 1	1 1 1	11 14 16	18 17 20	St St St	+++	Rabies Rabies Rabies						

St = Sterile,

TABLE IV  
Monthly Mean and Median Deaths from Smallpox in the period 1896 to 1922

Provinces	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
M <sub>1</sub>	746	847	1,009	889	747	626	621	525	416	379	396	585
	481	559	641	576	539	488	544	450	375	333	331	462
M <sub>2</sub>	579	577	654	614	586	509	537	547	492	483	485	546
	537	540	479	475	485	406	478	495	420	375	433	517
M <sub>3</sub>	578	579	610	556	481	432	467	454	463	486	462	553
	511	519	511	483	369	336	383	405	442	406	406	464
B <sub>y</sub>	477	703	937	837	564	329	216	129	86	73	117	241
	378	547	811	703	445	277	183	126	83	58	98	173
P <sub>F</sub>	1,234	1,115	1,220	1,295	1,679	1,526	1,135	606	363	321	543	980
	654	569	590	720	954	927	750	379	244	226	383	508
U <sub>P</sub>	746	846	1,402	2,248	2,424	1,892	1,228	550	228	125	214	511
	186	177	288	486	475	585	386	175	109	40	84	133
C <sub>P</sub>	324	380	539	633	608	480	328	219	121	89	115	209
	307	292	475	442	495	421	268	179	86	65	75	161

TABLE II  
Showing the effect of exposing 'Street Varus' cords to ether vapour for periods varying from 5 to 144 hours

ETHERISED CORDS		INOCULATION							REMARKS	SUB-PASSAGE			REMARKS
No of hours exposed.	No of cords exam-ined.	Sample taken from	No of rabbits inocu-lated.	Deaths	No of days to		Cerebro-spinal fluid	Negri-bodies					
					First symp-toms	Death							
5	1	Outside Inside Mixture	1 1 1	1	22	27	St	+	Rabies Alive 15 months after-wards Alive 9 months after-wards	No of rabbits sub-pas-saged	No of days to First symp-toms Death		
6	1	Outside Inside Mixture	1 1 1	1	13	17 1 114	St	+	Rabies Not rabies Not rabies				
7	1	Outside Inside Mixture	1 1 1	1 1 1	14 12 12	17 16 15	St St St	+ + +	Rabies Rabies Rabies				
8	1	Outside Inside Mixture	1 1 1	1 1 1	12 6 12	15 7 16	St St St	+ + +	Rabies Rabies Rabies				
9	1	Outside Inside Mixture	1 1 1	1 1 1	11 11 11	15 19 13	St St St	+ + +	Rabies Rabies Rabies				

St = Sterile

Taking a general survey of the disease during recent years, it may be said that a severe type of smallpox predominates in India, but the total mortality from smallpox is nowhere as high as that from cholera. Considerable differences in case mortality rates occur in different parts of the country, and these cannot altogether be ascribed to variations in the accuracy of notifications. During 1927, for example, it is reported that 'the case mortality rate on the basis of reported cases and deaths was very high (about 40 per cent) in Northern India (the Punjab and the United Provinces), somewhat lower in Bengal, Bihar and Orissa and Bombay Presidency, but in Madras Presidency and in the Central Provinces only one-third as high as in Northern India' (10). The case mortality rate for India as a whole, in the same year, was about 24 per cent, a figure corresponding with that of other countries of the Far East. 'The rates in 1926 were 29.6 per cent in Iraq, 20.3 per cent in Egypt, 35.6 per cent in Siam, 23.5 per cent in Korea and 13.8 per cent in Japan, where vaccination is more completely enforced' (10).

#### STATISTICS OF MORTALITY FROM SMALLPOX IN INDIA DURING THE PERIOD 1896 TO 1922

Although chicken-pox is mistaken for smallpox in a large number of cases, the diagnosis of smallpox in India may be taken for all practical purposes to be sufficiently accurate in so far as the mortality statistics are considered. In the case of such a well-known and widespread disease, mortality statistics are much more complete than morbidity figures, and no reluctance, therefore, need be felt in using the mortality data from 1866 onwards. For the sake of comparison with the cholera statistics, however, the data from 1896 only have been used.

The data have been classified according to the same 13 statistical areas as were used in the case of the cholera studies. The crude data, relating to the period 1896 to 1922, are shown in Table I.

It is obvious that smallpox is in constant evidence in all areas, although the degree of virulence is perhaps less in B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and B<sub>4</sub>, where almost 'free' years occasionally follow epidemic years. For example, in B<sub>1</sub>, 16,490 deaths occurred in 1909, and only 276 deaths in 1912, 639 in 1913 and 692 in 1914. In other areas, on the other hand, the annual incidence is consistently high with series of highly epidemic years. The statistical areas may, therefore, be grouped meantime under two heads (i) Group I. All areas except Bengal, (ii) Group II. Bengal Presidency.

In order to present a clear picture of the mortality in the different areas, the maximum and minimum deaths by months, over the period of years considered, are given in Tables II and III. The maximum incidence is very high in U P and P<sub>r</sub>, low in Bengal, C P and A, and only moderately high in other areas. The minimum incidence is uniformly high in the Madras Groups, B<sub>v</sub>, P<sub>r</sub>, B and O and perhaps also in A, whereas it is very low in Bengal, U P and C P. These radical differences would indicate that, in Bengal, the disease exists at times only in sporadic form, as is suggested by Table I.

84	1	Outside Inside Mixture	1 1 1	1 1 1	15 15 16	17 17 20	St St St	+++	Rabies Rabies Rabies				
96	1	Outside Inside Mixture	1 1 1	1	7	8	St	+	Alive after 150 days Rabies Alive after 150 days				
120	1	Outside Inside Mixture	1 1 1	1 1 1		5 16	Con St	- +	Alive after 149 days No sub-passage Rabies				
144	1	Outside Inside Mixture	1 1 1	1		98	Con	-	Not rabies Alive after 128 days Alive after 128 days				

Con = Contaminated

St = Sterile



# AN INVESTIGATION INTO THE VALUE OF AN ETHERISED VACCINE IN THE PROPHYLACTIC TREATMENT OF RABIES

## Part IV.

### THE ACTION OF ETHER ON THE VIRUS FIXÉ OF THE PASTEUR INSTITUTE, PARIS

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As the results obtained with the Kasauli fixed virus (Cunningham, Nicholas, Laluri, 1926) proved to be so different from those reported by European observers, we considered it advisable, if possible, to repeat the tests with a standard European virus so as to make certain that the variation was really due to a want of resistance on the part of the Kasauli virus itself and not to some difference in technique

For this purpose we obtained, through the kindness of M Calmette, a sample of the fixed virus in use at the Pasteur Institute, Paris. This virus, which is the virus originally fixed by M Pasteur himself, was in its 1,297th passage when it reached us and was used for the tests between the 1,298th and 1,307th passage. The technique employed was the same as that recorded in our original paper. The resistance of the virus in both brains and cords was tested. The brains and cords under test were kept in the ice-chest throughout the period of immersion at a temperature which varied between 10°C and 18°C. The results of the experiments are given in the two accompanying tables.

Remlinger (1919) experimenting with the virus fixé of the Pasteur Institute, Paris (presumably the virus at present under test), found that, in rabbits and



endemic foci Any inference drawn therefrom would not, however, necessarily represent the actual spread of smallpox in any given year but would merely show the tendency of spread on the average

TABLE V

*Statement showing the density of Smallpox Deaths in each Province*

Provinces	Mean	Median	Population in 1921	DEATH-RATE PER 100,000 POPULATION		REMARKS
				Mean	Median	
M <sub>1</sub>	7,786	5,779	13,102,293	59	44	Endemic.
M <sub>2</sub>	6,609	5,640	14,191,300	46	40	Do
M <sub>3</sub>	6,119	5,235	9,050,358	67	58	Do
M <sub>1</sub> +M <sub>2</sub> +M <sub>3</sub>	20,514	16,654	36,343,951	56	46	Do
B <sub>1</sub>	4,709	3,882	26,701,148	18	15	Epidemic.
P <sub>1</sub>	12,017	6,904	25,761,500	46	27	Endemic
U P	12,414	3,124	45,375,787	27	7	Epidemic.
C P	4,045	3,266	13,912,760	29	24	Do
B & O	12,053	10,580	34,002,189	35	31	Endemic
A	3,138	2,791	7,606,230	41	37	Do
B <sub>1</sub>	3,470	1,599	10,345,664	34	15	Epidemic.
B <sub>2</sub>	2,584	1,226	9,461,395	27	13	Do
B <sub>3</sub>	3,467	2,797	18,837,835	19	15	Do
B <sub>4</sub>	4,150	2,265	8,050,642	52	28	Endemic
B <sub>1</sub> +B <sub>2</sub> +B <sub>3</sub> +B <sub>4</sub>	13,671	7,887	46,695,536	29	17	Epidemic

## SEASONAL PERIODICITY

Graphs I to XIII have been prepared to show that smallpox, like cholera, has a seasonal periodicity The continuous lines represent average monthly smallpox deaths over the period 1896 to 1922, and the average monthly rainfall is shown by dotted lines The graphs show that in most areas smallpox ordinarily reaches its maximum incidence before the rains and begins to diminish after their commencement In M<sub>2</sub> and M<sub>3</sub>, however, this rule does not hold good

TABLE 1  
Showing the effect of immersing brains infected with the *Virus Fixé* of the Pasteur Institute, Paris, in ether for periods varying from 72 to 168 hours

THERISED BRAINS		INOCULATION					REMARKS	Cerebro spinal fluid	SUB-PASSAGE			Cerebro spinal fluid	REMARKS	
No of hours immer sed	No of brains trea- ted	Sample taken from	No of rabbits inocu lated	Deaths	No of days to				No of rabbits sub pas saged	No of days to				Death.
					First symp toms	Death				First symp toms	Death.			
72	1	Outside Inside Mixture	1 1 1	1 1 1	10 10 9	13 12 11	1 1 1	8 8 8	9 11 11	St St St	Rabies Rabies Rabies			
84	1	Outside Inside Mixture	1 1 1	1 1 1	10 9 9	11 11 11	1 1 1	7 8 8	8 10 10	St St St	Rabies Rabies Rabies			
96	1	Outside Inside Mixture	1 1 1	1 1 1	11 10 10	12 12 12	1 1 1	8 8 8	10 10 10	St St St	Rabies Rabies Rabies			
120	1	Outside Inside Mixture	1 1 1	1 1 1	11 10 10	13 13	1 1	8 8	9 9	St St	Rabies, Rabies			
144	1	Outside Inside Mixture	1 1 1	1 1 1	11 12 12	14 15 16	1 1 1	8 7 7	13 13 10	St St St	Rabies Rabies Rabies			
168	1	Outside Inside Mixture	1 1 1								• • •			

St = Sterile

TABLE VI—*contd*

Provinces	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
Mean	1,234	1,115	1,220	1,295	1,679	1,526	1,135	606	363	321	543	980	12,017
Percentage	10.3	9.3	10.2	10.8	14.0	12.7	9.4	5.0	3.0	2.7	4.5	8.1	100
$P_F$ Median	654	569	590	720	954	927	750	379	244	226	383	508	6,904
Percentage	9.5	8.2	8.5	10.5	13.8	13.4	10.9	5.5	3.5	3.3	5.5	7.4	100
Mean	746	846	1,402	2,248	2,424	1,892	1,228	550	228	125	214	511	12,414
Percentage	6.0	6.8	11.3	18.2	19.6	15.2	9.9	4.4	1.8	1.0	1.7	4.1	100
$U_P$ Median	186	177	288	486	475	585	386	175	109	40	84	133	3,124
Percentage	5.9	5.7	9.2	15.6	15.2	18.7	12.4	5.6	3.5	1.3	2.7	4.2	100
Mean	324	380	539	633	608	480	328	219	121	89	115	209	4,045
Percentage	8.0	9.4	13.3	15.7	15.0	11.9	8.1	5.4	3.0	2.2	2.8	5.2	100
$C_P$ Median	307	292	475	442	495	421	268	179	86	65	75	161	3,266
Percentage	9.4	9.0	14.5	13.5	15.2	12.9	8.2	5.5	2.6	2.0	2.3	4.9	100
Mean	888	1,009	1,596	1,990	1,943	1,551	966	573	318	235	336	648	12,053
Percentage	7.4	8.4	13.2	16.5	16.1	12.9	8.0	4.8	2.6	1.9	2.8	5.4	100
$B \& O$ Median	832	1,082	1,337	1,445	1,536	1,474	877	599	321	225	345	507	10,580
Percentage	7.9	10.2	12.6	13.7	14.5	13.9	8.3	5.7	3.0	2.1	3.3	4.8	100

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The following statement gives the months of maximum and minimum incidence for each area —

Areas	Maximum Incidence	Minimum Incidence
M <sub>1</sub>	March	October
M <sub>2</sub>	March	October
M <sub>3</sub> .	March	June and November
B <sub>y</sub> .	March	October
P <sub>r</sub> .	May	October
U P	May	October
C P . ..	April	October
B & O ..	April	October
A ..	May	October
B <sub>1</sub> . ..	May	October
B <sub>2</sub> .. ..	March	October
B <sub>3</sub> .. ..	April	October
B <sub>4</sub> . ..	March	October

It is evident that, in nearly every area, a continuous fall occurs from the maximum to a minimum in October

In M<sub>1</sub>, the incidence of smallpox is highest between February and April, and falls rapidly with the onset of the south-west monsoon rains. A comparatively low figure continues throughout the rainy season with a rise again at the end of the year. The average monthly mortality never falls below 350, whilst the maximum may exceed 1,000.

In M<sub>2</sub>, the monthly incidence remains comparatively high, even during the rains, the highest and lowest monthly averages lying between 700 and 450.

In M<sub>3</sub>, although both monsoons are experienced, the incidence is very similar to that in M<sub>2</sub>.

In B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>, the annual wave of mortality ordinarily commences in March with an average of 400 deaths, reaches a maximum in May, and by August falls to less than 150, this low figure continuing until the end of the year. In B<sub>4</sub>,

# AN INVESTIGATION INTO THE VALUE OF AN ETHERISED VACCINE IN THE PROPHYLACTIC TREATMENT OF RABIES

## Part V.

### THE ACTION OF ETHER ON A FRESHLY FIXED VIRUS OF INDIAN ORIGIN

BY

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*(Investigation aided by a grant from the Indian Research Fund Association)*

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THE widely varying results obtained by us (Cunningham, Nicholas and Lahiri, 1926, 1927, 1928) with the Kasauli fixed virus and the virus fixé obtained from the Pasteur Institute, Paris, have raised the question whether other strains of fixed virus exhibit similar differences in their resistance to the action of ether. We have, therefore, considered it advisable to test the behaviour of an entirely new strain which we have fixed expressly for this purpose.

This virus known as Kasauli fixed virus No 2 (K F V No 2) originated from a rabid dog killed in Kasauli on 1st March, 1926. The first sub-passage into a rabbit showed symptoms in 10 days and death on the 12th day. Negri-bodies disappeared, and the virus appeared fixed, by the 16th passage, symptoms appearing in 9 days and death in 11 days. It was used for this experiment after its 38th passage when symptoms were occurring on the 7th and death on the 9th day. In this respect, therefore, it showed the characteristics of the original Kasauli fixed virus (K F V No 1) rather than those of the 'Paris' fixed virus. The resistance of the virus in infected brains alone was tested. The

the partial correlation work, tables previously prepared were also used, and as a result, the computations were successfully completed in a very short time. No excuse is made for drawing attention once more to the advantages of this 'short method,' and the hope is confidently expressed that other workers will find it equally useful.

Table VII gives complete sets of coefficients between smallpox mortality and the four climatic factors for  $\text{lag}_0$  and for lags of one and two months.

As the total frequency for most areas is the same, viz., 313, and the coefficients of each area are compared with those of other areas, a coefficient is taken as statistically significant if its absolute value exceeds 0.15, since for this value of 'r' the probable error is only  $\pm 0.0362$ . In view of this fact, and in order to save space, probable errors are not given. In  $M_2$  and  $M_3$ , where the total frequency is less in respect of the pressure factor, the highest probable error has been taken into consideration. The only correlation tables included in this paper are those for relative humidity and smallpox.

#### RAINFALL

Table VII shows that, excluding the coefficients for  $M_2$  and  $M_3$  and the  $\text{lag}_0$  coefficients for A and  $B_1$ , a negative association exists between smallpox and rainfall all over India, and a lag of two months is evident in most areas. In  $M_2$  and  $M_3$ , the coefficients are all negative and insignificant. It might be said indeed that, with the single exception of  $\text{lag}_0$  for A, every coefficient is negative with respect to rainfall. This is a very important finding because it shows clearly that rainfall has an inhibitory effect on the virulence of smallpox. No such uniform deduction was possible in the case of cholera.

#### RELATIVE HUMIDITY

The coefficients for  $\text{lag}_0$ ,  $\text{lag}_1$  and  $\text{lag}_2$  are all negative and uniformly significant in every area, except  $M_2$  and  $M_3$ . Whilst the coefficients for  $M_2$  are insignificant, however, those for  $M_3$  are all positive and significant. In the case of this climatic factor, the lag is not so evident, since little variation is to be noted between the three coefficients for each area, but in most cases a lag of one month might be inferred. The comparatively high values of the coefficients show that high relative humidity is opposed to an increased virulence of smallpox, and that the disease is unlikely to assume a virulent form under conditions of high relative humidity.

#### TEMPERATURE

All the coefficients in  $M_3$  are significant and negative, but for other areas no general conclusion can be drawn.  $\text{Lag}_0$  coefficients are positive and significant only in B & O and C P, whilst those for other areas are insignificant. Since  $\text{lag}_2$  coefficients are all negative, and are significant in nearly every area, a general two months' lag seems to be indicated.

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# THE EPIDEMIOLOGY OF SMALLPOX

BY

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## INTRODUCTION

IN a series of papers (2 to 6) on the Epidemiology of Cholera, the rôle of the climatic factors, rainfall, relative humidity, temperature and pressure on the incidence and spread of cholera has been discussed in detail

In the case of smallpox, there also exists the need for examination of the circumstances which, at intervals, give to this endemic disease a new power of dissemination, enabling it periodically to exercise a far more destructive influence at one time than another. What are the circumstances which favour the spread of this disease? Given other identical conditions, do climatic factors have any causal relationship with its spread in different areas? The attempt is here made to answer these questions in so far as the climatic conditions of the atmosphere are concerned

## HISTORY OF SMALLPOX

It is unnecessary to give any details of the history of smallpox, since it has been a familiar disease from the earliest times. 'Smallpox takes the first place among epidemic diseases in the tenacity and malignity with which it has pursued the human race over all the world, and the tale of the destructive ravages of this pestilence in early times and of the suffering which resulted from it fills the mind with horror' (9)

It was prevalent in China at least 2,000 years ago and in India it has existed as far back as records go. In Europe, smallpox appears to have reached its highest point of intensity and distribution in the eighteenth century, the mortality in England alone being one-tenth of the total in Europe

As regards the civil population of India, the era of medical statistics did not commence until 1866, and exact information regarding the prevalence of smallpox before that time is very scanty. Ancient temples dedicated to the goddess of smallpox are proof, however, that from the earliest times few parts of the country escaped this pestilence

different provinces, smallpox seems to be more or less uniformly influenced by climate in all areas, except perhaps in the south-east of Madras Presidency

Ignoring pressure correlations for the time being, the other three climatic factors are all negatively associated with smallpox, i.e., each has inhibitory influence on the virulence of the disease. Moreover, the coefficients are uniform in all areas, except  $M_2$  and  $M_3$ , and this indicates that smallpox is an endemic disease and does not necessarily arise in special tracts, although an epidemic might start in one locality with more virulence than in another. The following paragraphs will attempt to explain why this is so.

#### PARTIAL CORRELATIONS OF THE THIRD ORDER

Before coming to a final conclusion, it is necessary to examine the partial correlation coefficients in order to determine the effect of each climatic factor when the others are held constant either singly or in combination.

Table IX (*see Appendix*) gives the coefficients of all orders for every area and these are re-arranged in Table X (*see Appendix*) to show their progressive variations from zero to third order. Each coefficient has been computed by two different paths, as a check.

The third order coefficients given in Table XI may be considered first.

TABLE XI

Areas	$r_{12\ 345}$	$r_{13\ 245}$	$r_{14\ 235}$	$r_{15\ 234}$
$M_1$	+0.0732	-0.1748	+0.0120	+0.1033
$M_2$	+0.1636	-0.2408	+0.1444	+0.3125
$M_3$	-0.0594	-0.0052	-0.0948	+0.0065
$B_v$	+0.0789	-0.3150	+0.0848	+0.0107
$P_F$	+0.0976	-0.1758	-0.1096	-0.0532
U P	+0.0406	-0.2093	-0.1376	-0.1803
C P	+0.2153	-0.3450	+0.0769	+0.0607
B & O	-0.0507	-0.3063	-0.0796	-0.2443
A	+0.1707	-0.3792	-0.2677	-0.1251
$B_1$	-0.0041	-0.2324	-0.0609	-0.1326
$B_2$	+0.0399	-0.2611	+0.0150	+0.0311
$B_3$	+0.1574	-0.3825	-0.0638	-0.0062
$B_4$	-0.0139	-0.1781	+0.0371	-0.0170

Subscript 1 = smallpox

„ 2 = rainfall

„ 3 = relative humidity

„ 4 = temperature

„ 5 = pressure

TABLE III  
Minimum number of Deaths from Smallpox in the period 1896 to 1922

Areas	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
M <sub>1</sub>	234	210	209	210	181	241	188	205	212	184	143	217
M <sub>2</sub>	74	76	100	77	73	88	89	76	65	74	89	89
M <sub>3</sub>	131	162	148	106	149	116	103	144	150	145	137	27
B <sub>Y</sub>	116	104	179	187	111	67	63	27	19	17	28	47
P <sub>Y'</sub>	64	63	91	85	187	165	159	114	82	68	50	54
U P	9	16	22	41	37	32	31	12	7	2	11	12
C. P	13	11	28	36	53	39	23	9	3	0	7	8
B & O	120	155	285	420	378	244	140	72	63	55	70	91
A	40	34	82	58	123	116	67	53	11	24	17	47
B <sub>1</sub>	6	6	10	14	19	13	2	1	6	1	3	5
B <sub>2</sub>	12	17	41	56	27	22	6	1	8	2	4	7
B <sub>3</sub>	13	23	26	46	26	26	14	5	4	7	13	10
B <sub>4</sub>	21	24	39	30	22	18	23	13	11	3	2	17

TABLE XII

Province	r12.345		r13 245		r14 235		r15.234	
	Lag <sub>0</sub>	Lag <sub>1</sub>	Lag <sub>0</sub>	Lag <sub>1</sub>	Lag <sub>0</sub>	Lag <sub>1</sub>	Lag <sub>0</sub>	Lag <sub>1</sub>
M <sub>1</sub>	+0.0732	+0.1580	-0.1748	-0.2560	+0.0120	-0.1205	+0.1033	+0.1975
M <sub>2</sub>	+0.1636	+0.2364	-0.2408	-0.3290	+0.1444	-0.0474	+0.3125	+0.2937
M <sub>3</sub>	-0.0594	+0.0466	-0.0052	-0.0605	-0.0948	-0.2034	+0.0065	-0.0327
B <sub>V</sub>	+0.0789	+0.1001	-0.3150	-0.1980	+0.0848	-0.2653	+0.0107	+0.0765
P <sub>F</sub>	+0.0976	+0.1894	-0.1758	-0.2947	-0.1096	+0.0005	-0.0532	+0.1814
U P	+0.0406	+0.1647	-0.2093	-0.3688	-0.1376	-0.3267	-0.1803	-0.1746
C P	+0.2153	+0.1847	-0.3450	-0.3920	+0.0769	-0.1596	+0.0607	+0.1214
B & O	-0.0507	+0.1897	-0.3063	-0.5097	-0.0796	-0.3335	-0.2443	-0.0114
A	+0.1707	+0.0784	-0.3792	-0.4602	-0.2677	-0.3284	-0.1251	-0.0194
B <sub>1</sub>	-0.0041	+0.0728	-0.2324	-0.3717	-0.0609	-0.1779	-0.1326	-0.0637
B <sub>2</sub>	+0.0399	+0.1162	-0.2611	-0.2851	+0.0150	-0.1525	+0.0311	+0.0565
B <sub>3</sub>	+0.1574	+0.1632	-0.3825	-0.3921	-0.0638	-0.1917	-0.0062	+0.1041
B <sub>4</sub>	-0.0139	+0.0467	-0.1781	-0.1726	+0.0371	-0.0608	-0.0170	+0.0796

Consideration of both sets of coefficients in Table XII makes it clear that humidity alone has a uniformly steady mitigating influence on the virulence of smallpox. On the other hand, the association of rainfall with virulence of smallpox appears to increase after a period of about two months.

#### RELATIVE INFLUENCE OF EACH CLIMATIC FACTOR ON THE ASSOCIATION OF THE OTHERS WITH THE VIRULENCE OF SMALLPOX

Table XIII (see Appendix)

Only partial coefficients derived from lag<sub>0</sub> coefficients will be considered in this connection.

#### Northern Districts Group, Madras Presidency (M<sub>1</sub>)

*Rainfall* does not affect the associations of humidity or temperature with smallpox. It has a slight effect on the association of pressure with smallpox.

*Humidity* affects the associations of rainfall and temperature with smallpox. It affects the association of pressure with smallpox only slightly.

*Temperature* does not affect the associations of rainfall and humidity with smallpox, but slightly affects that of pressure with smallpox.

TABLE IV—contd

Provinces	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec.
B & O	888	1 009	1,596	1,990	1,943	1 551	966	573	318	235	336	648
	832	1,082	1,337	1,445	1,536	1,474	877	599	321	225	345	507
A	232	265	362	429	478	391	276	175	124	96	119	191
	196	201	267	387	420	348	302	170	106	100	107	187
B <sub>1</sub>	166	224	424	615	703	537	307	161	90	57	67	119
	79	130	169	247	288	276	162	70	42	33	44	59
B <sub>2</sub>	265	365	516	466	325	198	116	70	46	38	51	128
	100	98	162	264	197	135	59	50	33	28	42	58
B <sub>3</sub>	275	352	533	575	491	376	246	141	115	85	93	185
	193	245	437	579	434	281	179	112	78	59	64	136
B <sub>4</sub>	337	459	740	728	601	404	245	140	104	79	89	224
	167	232	438	352	327	245	148	77	50	45	69	115

*The United Provinces of Agra and Oudh (U P)*

*Rainfall* does not affect any of the associations of humidity, temperature or pressure

*Humidity* affects the associations of rainfall and temperature, but not that of pressure

*Temperature* has no effect on the associations of rainfall, humidity or pressure

*Pressure* affects the association of rainfall slightly It has no effect on the association of humidity but affects that of temperature

*Central Provinces and Berar (C P)*

*Rainfall* does not affect the association of humidity It affects the association of temperature when humidity is constant, and also affects that of pressure

*Humidity* affects the association of rainfall greatly It also affects those of temperature and pressure

*Temperature* does not affect the associations of rainfall or humidity, but affects that of pressure

*Pressure* affects the association of rainfall when humidity is not constant It does not affect the association of humidity, or that of temperature when humidity is not constant

*Bihar and Orissa (B & O)*

*Rainfall* does not affect the association of humidity, but affects those of temperature and pressure

*Humidity* affects the associations of rainfall and pressure It affects that of temperature only when rainfall is constant

*Temperature* affects the associations of rainfall and pressure to some extent, but not that of humidity

*Pressure* affects the associations of rainfall and temperature, but not that of humidity

*Assam (A)*

*Rainfall* has no effect on the association of humidity When humidity is held constant, it affects the association of temperature It also affects that of pressure

*Humidity* affects the association of rainfall, and, when rainfall is constant, that of temperature It has no effect on that of pressure

*Temperature* has no effect on the association of rainfall, except when humidity is held constant It has no effect on the association of humidity, but affects that of pressure

1

1

2



The influence of the various climatic factors may be summed up in tabular form as follows —

Provinces	EFFECT OF			
	Rainfall on the association of smallpox with	Humidity on the association of smallpox with	Temperature on the association of smallpox with	Pressure on the association of smallpox with
M <sub>1</sub>	.	* R. T		T
M <sub>2</sub>			P	H <sub>g</sub> R. T
M <sub>3</sub>	..		P H (P <sub>nc</sub> )	T H (T <sub>nc</sub> )
B <sub>Y</sub>	P	R.		
P <sub>F</sub>				
U P		R. T		T
C P	P	R <sub>g</sub> T P	P	
B & O	T P	P		R T
A	P	R.	P	R. T
B <sub>1</sub>		R.		R. T
B <sub>2</sub>		R.		
B <sub>3</sub>		R.		
B <sub>4</sub>		R.		

\* "R" = Significant Association

"R<sub>g</sub>" = Very Significant Association

"P<sub>nc</sub>" = When "P" is not constant

The following inferences can be made from the table —

1 The association of humidity with smallpox in M<sub>2</sub> is affected to a high degree by pressure, and this association is also affected to some extent by both temperature and pressure in M<sub>3</sub>. In all other areas, the association of humidity with smallpox is unaffected by any of the other factors

2 Humidity affects the association of rainfall with smallpox in all areas except B & O, P<sub>F</sub>, M<sub>2</sub> and M<sub>3</sub>. In addition, humidity affects the association of temperature with smallpox in U P, C P and M<sub>1</sub>

3 Pressure affects the association of rainfall with smallpox in B & O, A, B<sub>1</sub> and M<sub>2</sub>, and that of temperature with smallpox in B & O, A, B<sub>1</sub>, U P, M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub>

4 Rainfall affects pressure associations with smallpox in B<sub>1</sub>, C P, B & O and A

5 Temperature affects the associations of pressure with smallpox in A, C P, M<sub>2</sub> and M<sub>3</sub>.

The monthly mean and median deaths, shown in Table IV, give a more exact idea of the incidence in each area. Since acute exacerbations, so frequently met with in the case of cholera, practically never occur with smallpox, it follows that either the mean or the median represents the monthly average number of deaths in the period considered. The differences between mean and median deaths, therefore, represent real differences in the incidence of the disease. If the median is low compared with the mean, as in  $B_1$  or  $B_2$ , it follows that the mortality is normally low in those areas, although they may be subject to occasional severe epidemics. On the other hand, if mean and median are high and nearly equal, as in the Madras groups, B & O and  $P_r$ , the inference is that the average incidence is high, and that these areas are uniformly affected by severe outbreaks, or, in other words, that the areas are endemic. From this argument, it may be deduced perhaps that smallpox is ordinarily sporadic in Bengal, that it is usually highly epidemic in U P, that it is less severely epidemic in C P and  $B_r$ , and that  $M_1$ ,  $M_2$ ,  $M_3$ , B & O,  $P_r$  and A are endemic areas for the disease.

By computing the mean and median death-rates per 100,000 of the population (Table V), the different areas can also be roughly classified as endemic or epidemic. Under endemic, may be included all three groups of Madras Presidency,  $P_r$ , B & O, A and  $B_4$ , the remaining areas suffer from epidemics of greater or less severity.

Table VI gives the monthly percentages of the annual mean and median mortality for each area. This table shows that the periods of comparatively high incidence of smallpox in each area are as follows —

<i>Area</i>	<i>Period</i>
$M_1$	December to July
$M_2$	. December to December
$M_3$	. December to December
$B_r$	January to June
$P_r$	December to July
U P	March to July
C P	March to June
B & O	January to June
A	March to July
$B_1$	March to July
$B_2$	January to June
$B_3$	February to June
$B_4$	January to June

These preliminary results might possibly be used to indicate, in approximate fashion, the probable spread of epidemics of smallpox from the different

A single + or — sign indicates only slight association, hence the only factors that need be considered with reference to smallpox are —

Areas	Factors having important bearing on the incidence of smallpox
M <sub>1</sub>	Humidity
M <sub>2</sub>	Pressure
M <sub>3</sub>	Nil
B <sub>1</sub>	Humidity
P <sub>F</sub>	Humidity
U P	Humidity and pressure
C P	Humidity and rainfall
B & O	Humidity and pressure
A	Humidity, temperature and rainfall
B <sub>1</sub>	Humidity
B <sub>2</sub>	Humidity
B <sub>3</sub>	Humidity and rainfall
B <sub>4</sub>	Humidity

Humidity is a common factor in all areas, except M<sub>2</sub> and M<sub>3</sub>, where as shown previously the association of humidity with smallpox is affected by temperature and pressure. Further, in all areas, the influence of humidity on smallpox is primary, except in M<sub>2</sub>, where pressure has a great effect on that association. Rainfall is of secondary importance in A, B<sub>3</sub>, and C P.

MULTIPLE CORRELATION

Coefficients of multiple correlation for the different areas have been computed, and are as follows —

Areas	R <sub>1 2345</sub>
M <sub>1</sub>	0 2929
M <sub>2</sub>	0 3372
M <sub>3</sub>	0 1536
B <sub>1</sub>	0 4010
P <sub>F</sub>	0 1884
U P	0 3239
C P	0 4432
B & O	0 5267
A	0 4494
B <sub>1</sub>	0 3158
B <sub>2</sub>	0 3139
B <sub>3</sub>	0 3869
B <sub>4</sub>	0 2236

The coefficients are less than 0 29 in three areas only, viz, M<sub>3</sub>, P<sub>F</sub>, and B<sub>4</sub>, but in the case of cholera, they were in every case but one greater than 0 4. This difference is perhaps due to the fact that whereas in the latter case more than one climatic factor had an effect, in smallpox, relative humidity alone has been shown to have any significance. Since in endemic areas, the effect of a disturbing factor need not necessarily be high, it may be inferred that in these

TABLE VI  
Mean and Median Deaths by months for each Province and the percentage of each month to the total

Provinces	Jan	Feb	March	April	May	June	July	Aug	Sept.	Oct.	Nov	Dec.	Total
Mean	746	847	1,009	889	747	626	621	525	416	379	396	585	7,786
Percentage	9.6	10.9	13.0	11.4	9.6	8.0	8.0	6.7	5.3	4.9	5.1	7.5	100
M, Median	481	559	641	576	539	488	544	450	375	333	331	462	5,779
Percentage	8.3	9.7	11.1	10.0	9.3	8.4	9.4	7.8	6.5	5.8	5.7	7.8	100
Mean	579	577	654	614	586	509	537	547	492	483	485	546	6,609
Percentage	8.8	8.7	9.9	9.3	8.9	7.7	8.1	8.3	7.4	7.3	7.3	8.3	100
M, Median	537	540	479	475	485	406	478	495	420	375	433	517	5,640
Percentage	9.5	9.5	8.5	8.5	8.6	7.2	8.6	8.7	7.4	6.6	7.7	9.2	100
Mean	578	579	610	556	481	432	467	454	463	486	462	553	6,119
Percentage	9.4	9.5	10.0	9.1	7.9	7.1	7.6	7.4	7.6	7.9	7.5	9.0	100
M, Median	511	519	511	483	369	336	383	405	442	406	406	464	5,235
Percentage	9.8	9.9	9.8	9.2	7.0	6.4	7.3	7.7	8.4	7.8	7.8	8.9	100
Mean	477	703	937	837	564	329	216	129	86	73	117	241	4,709
Percentage	10.1	14.9	19.9	17.8	12.0	7.0	4.6	2.7	1.8	1.6	2.5	5.1	100
M, Median	373	547	811	703	445	277	183	126	83	58	98	173	3,882
Percentage	9.7	14.1	20.9	18.2	11.5	7.1	4.7	3.2	2.1	1.5	2.5	4.5	100

warnings and to organize additional vaccination staff to cope with and minimize the approaching outbreak of smallpox'

These statements, originating as they do from such an eminent writer, demand detailed examination

As regards (iii), it is customary to use relative humidity figures in epidemiological investigations, as obviously it is the relative rather than the absolute amount of water vapour that really matters in organic growth. Moreover, it is by no means clear what is meant by the 'close relation' between smallpox prevalence and absolute humidity

As regards (iv), Table XV shows that the average monthly absolute humidity is uniformly high in  $M_2$  and  $M_3$ , and is higher in those areas than in any other part of India during the months of November to May. Yet it is these very areas that suffer most from smallpox, whilst in C P, where a very low absolute humidity occurs in most months, smallpox is not endemic throughout the year as it is in  $M_2$  and  $M_3$ . Table IV giving the monthly mean number of deaths from smallpox in different areas, taken in conjunction with Table XV, demonstrates these facts very clearly

Again, as regards (vi), the periods with less than an average of 0.700 and 0.800 absolute humidity are tabulated below with the periods of average high smallpox mortality

*Absolute Humidity and Smallpox*

Areas	Period of $\angle 0.800$	Epidemic period	Period of $\angle 0.700$
$M_1$	October to April	December to July	November to March
$M_2$	June to March	December to December	December to January
$M_3$	November to March	December to December	December to January
$B_V$	August to May	January to June	October to April
$P_F$	September to June	December to July	October to June
U P	October to June	March to July	October to May
C P	August to June	March to June	October to May
B & O	October to May	January to June	November to April
A.	November to April	March to July	November to April
$B_1$	November to April	March to July	November to April
$B_2$	November to March	January to June	November to February
$B_3$	November to March	February to June	November to March.
$B_4$	November to April	January to June	November to March

This table shows clearly that the inference that forecasts of smallpox epidemics can be made by watching absolute humidities between 0.700 and 0.800 is by no means justified

A	Mean	232	265	362	429	478	391	276	175	124	96	119	191	3,138
	Percentage	7.4	8.4	11.5	13.7	15.2	12.5	8.8	5.6	3.9	3.1	3.8	6.1	100
	Median	196	201	267	387	420	348	302	170	106	100	107	187	2,791
	Percentage	7.0	7.2	9.6	13.9	15.0	12.5	10.8	6.1	3.8	3.6	3.8	6.7	100
B <sub>1</sub>	Mean	166	224	424	615	703	537	307	161	90	57	67	119	3,470
	Percentage	4.8	6.5	12.2	17.7	20.3	15.5	8.9	4.6	2.6	1.6	1.9	3.4	100
	Median	79	130	169	247	288	276	162	70	42	33	44	59	1,599
	Percentage	4.9	8.1	10.6	15.4	18.0	17.3	10.1	4.4	2.6	2.1	2.8	3.7	100
B <sub>2</sub>	Mean	265	365	516	466	325	198	116	70	46	38	51	128	2,584
	Percentage	10.3	14.1	19.9	18.0	12.6	7.7	4.5	2.7	1.8	1.5	2.0	4.9	100
	Median	100	98	162	264	197	135	59	50	33	28	42	58	1,226
	Percentage	8.2	8.0	13.2	21.5	16.1	11.0	4.8	4.1	2.7	2.3	3.4	4.7	100
B <sub>3</sub>	Mean	275	352	533	575	491	376	246	141	115	85	93	185	3,467
	Percentage	7.9	10.2	15.4	16.6	14.2	10.8	7.1	4.1	3.3	2.4	2.7	5.3	100
	Median	193	245	437	579	434	281	179	112	78	59	64	136	2,797
	Percentage	6.9	8.8	15.6	20.7	15.6	10.0	6.4	4.0	2.8	2.1	2.3	4.8	100
B <sub>4</sub>	Mean	337	459	740	728	601	404	245	140	104	79	89	224	4,150
	Percentage	8.1	11.1	17.8	17.6	14.5	9.7	5.8	3.4	2.5	1.9	2.2	5.4	100
	Median	167	232	438	352	327	245	148	77	50	45	69	115	2,265
	Percentage	7.4	10.2	19.3	15.5	14.5	10.9	6.5	3.4	2.2	2.0	3.4	4.7	100

On the other hand, the figures are overwhelmingly in favour of relative humidity. Table XVI gives the average monthly relative humidity in each area over the period 1896 to 1920. This table, taken in conjunction with the figures in Table IV, makes it clear that areas of low relative humidity suffer more from virulent smallpox than areas of high relative humidity such as the Bengal groups. As has already been shown,  $M_2$  and  $M_3$  are exceptional areas where smallpox has little or no association with humidity. Graphs XIV to XXVI, which represent smallpox and relative humidity monthly averages, also make it evident that in every area except  $M_2$  and  $M_3$ , smallpox becomes virulent when relative humidity is low and that epidemics are generally preceded by months of falling relative humidity, and vice versa.

On the other hand, Graphs XXVII to XXXIX, representing smallpox and absolute humidity monthly averages, prove that no such simple inference can be drawn, but that, as the period of rising smallpox incidence more or less coincides with the period of rising absolute humidity in nearly every area, the inverse relationship between absolute humidity and smallpox incidence suggested by Rogers does not exist.

#### CONCLUSION

Relative humidity is, therefore, the inhibitory factor and not absolute humidity, and this important result has been corroborated by every method of examination. It follows that, by noting changes in relative humidity, especially in dry areas, this indicator can be used to forecast epidemics of smallpox well in advance.

In  $M_2$  and  $M_3$ , little evidence exists of a relationship between lack of humidity and a rise in the incidence of smallpox, for, in these areas, the reason for the steady and exceptionally high incidence of smallpox is the absence or partial failure of the south-west monsoon, and the irregularity of the north-east monsoon which, in some years, is not only delayed but completely fails.

It is probable that low relative humidity consequent on conditions following failure of the periodic rains produces conditions favourable to the development of virulent smallpox.

#### SMALLPOX AND VACCINATION

Lack of humidity being thus associated with a rise in the virulence of smallpox, can Public Health Departments make any use of the fact?

The only effective weapon known to science in respect of the prevention of smallpox is vaccination. All countries where vaccination has been extensively carried out have given an unequivocal answer in favour of the practice, and this preventive measure, indeed, has been described as the 'greatest physical good ever given by science to the world'.

The introduction and progress of vaccination in India has always been attended with difficulties, and, as far back as 1804, Dr John Schoolbred published an interesting report on the subject. Although much has been done since

the monthly mortality curve is similar to those of  $B_1$ ,  $B_2$  and  $B_3$ , but the maximum is somewhat higher

The curves for A, U P, B & O, C P and  $B_v$  are very similar, although the average incidence is higher in some cases than in others

In  $P_r$ , the peak is reached in May, and the fall in incidence is of short duration, the average monthly figure rising again to a high level by December. A clearly marked annual periodicity, therefore, occurs in every province in India.

### PERIODICITY OF SMALLPOX

It has not been thought necessary to make periodogram analyses for every area, as was done in the case of cholera. Only six graphs are given, and these indicate that a seven-year cycle is probable.

In the case of the Madras groups, figures for the period 1866 to 1925 were used, those from 1870 to 1925 for Madras City and from 1896 to 1922 for C P and  $B_v$ .

In Graphs A, B and C for the three groups of the Madras Presidency and in Graph D for Bombay, a seven-year periodicity is indicated, whilst Graph E for C P shows a six or seven years' periodicity to be most probable. The figures for Madras City were also analysed by the same method, and it is interesting to note that in this restricted area a seven years' periodicity also occurs (Graph F).

Holwell, in 1767, writing of the prevalence of smallpox in Bengal, made the observation that — 'Every seventh year with scarcely any exception the smallpox rages epidemically during the months of March, April and May, and, sometimes, until the annual returning rains about the middle of June put a stop to its fury' (9). That Holwell's observation made 160 years ago should be capable of verification by modern statistical methods is remarkable. Rough as the method of periodogram analysis may be, it indicates trends which can be verified as further data accumulate. For the present, all that can be said is that smallpox seems to have a periodic trend of about seven years in the areas covered by these analyses.

### THE METHODS OF CORRELATION

As the main purpose of the present investigation is to ascertain the effect, if any, of climatic factors on the virulence of smallpox, simple and partial coefficients of correlation have been computed in the same manner as those for cholera. In the cholera work, owing to occasional acute and irregular exacerbations in the data, moving averages were used. No such correction was necessary in the case of smallpox, as the mortality figures for this disease are never very high or very irregular.

In the correlation work, the short method (8) was employed throughout, both labour and time being largely saved by using the same 'chambered' numbers for the climatic factors as were used in connection with cholera. Further, in



then, however, the incidence of smallpox is still considerably higher than it ought to be, and every effort must be made to extend protection by efficient vaccination, especially in the drier months of the year when smallpox tends to become more virulent, and in those areas now shown to be particularly endemic. Moreover, as it has been shown to be possible, by studying the variations in local weather conditions, to foretell the outbreak of smallpox epidemics, from now onwards this knowledge should be given practical application throughout the whole of India.

As a direct result of this study, the forecast has been made that smallpox will probably be virulent in Madras Presidency during the present year, and every Health Officer in the Province has been instructed to take all possible preventive measures. There is reason to believe that the forecast has been correct, and it remains to be seen if the timely warning has been of avail.

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\* These five papers have been published as a Memoir on 'The Epidemiology of Cholera in India' (*Ind Med Res Memoirs*, No 12, Oct 1928)

TABLE VII

	U P	C P	B & O	A	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>4</sub>
1296	—0·0524	—0·0770	—0·1302	+0·1678	+0·0163	—0·1550	—0·0128	—0·0953
739	—0·1406	—0·2768	—0·3469	—0·1166	—0·1362	—0·2563	—0·2141	—0·2161
'091	—0·1768	—0·3821	—0·4399	—0·3534	—0·2286	—0·3033	—0·3484	—0·2747
307	—0·2455	—0·3371	—0·4225	—0·3143	—0·2351	—0·3112	—0·3290	—0·2006
940	—0·2863	—0·4386	—0·5400	—0·3688	—0·3991	—0·3074	—0·4115	—0·2945
502	—0 2312	—0·4027	—0·4403	—0·2922	—0·3942	—0·2439	—0·3585	—0·2565
223	+0·1401	+0·2480	+0·2069	+0·0829	+0 1208	—0·0379	+0·0361	+0·0225
696	+0·0281	—0·0231	—0·0474	—0·1720	—0·0246	—0·2466	—0·1950	—0·1665
1452	—0·1028	—0·2201	—0·3106	—0·3954	—0·1902	—0·3810	—0·3775	—0·3157
291	—0·1671	—0·0773	—0·1895	—0·1893	—0·1457	+0·1064	—0 0168	+0·0149
1091	—0·0581	+0·1715	+0·0885	+0·0889	+0·0116	+0·2790	+0·2194	+0·2041
'131	+0·0488	+0·3810	+0·3237	+0 3336	+0·1606	+0·3919	+0·3990	+0·3401

M <sub>1</sub> S—H <sub>0</sub>	NUMBER OF DEATHS FROM SMALLPOX																Total		
	RELATIVE HUMIDITY IN PERCENTAGES																		
60—62	5	123	87	41	13	17	8	6	8	5	4	2	2	1		1	1	1	325
62—64																			
64—66					3	1	1			1							1		
66—68				7	4	1			4	1	3								
68—70				6	2	1	1			2				1					
70—72				9		1	3	1	1				2				1		
72—74				3		4	2	2	2		1								
74—76				5	1	3		2											
76—78				4	1	2	1		1										
78—80				4	1	2													
80—82				2	1	1				1									
82—84				2															
84—86				2															

## PRESSURE

The groups of coefficients for pressure are of much the same magnitude as those for temperature, but, with unimportant exceptions, are all opposite in sign. Lag<sub>1</sub> coefficients are positive when significant, whilst lag<sub>2</sub> coefficients are all positive and significant with the exception of that for U P. In this case, also, a lag of two months is indicated.

Since a lag effect is in evidence for each climatic factor, it has seemed worth while to make further examination of the lag coefficients.

TABLE VIII

Province	Rainfall (lag <sub>1</sub> )	Humidity (lag <sub>1</sub> )	Temperature (lag <sub>2</sub> )	Pressure. (lag <sub>2</sub> )
M <sub>1</sub>	-0.2927	-0.2429	-0.2493	+0.3778
M <sub>2</sub>	-0.0622	-0.0332	-0.1375	+0.1762
M <sub>3</sub>	+0.0556	+0.1907	-0.2671	+0.1875
B <sub>r</sub>	-0.3420	-0.4441	-0.5451	+0.5147
P <sub>r</sub>	-0.2091	-0.1940	-0.1452	+0.2131
U P	-0.1768	-0.2863	-0.1028	+0.0488
C P	-0.3821	-0.4386	-0.2201	+0.3810
B & O	-0.4399	-0.5400	-0.3106	+0.3237
A	-0.3534	-0.3688	-0.3954	+0.3336
B <sub>1</sub>	-0.2286	-0.3991	-0.1902	+0.1606
B <sub>2</sub>	-0.3033	-0.3074	-0.3810	+0.3919
B <sub>3</sub>	-0.3484	-0.4115	-0.3775	+0.3990
B <sub>4</sub>	-0.2747	-0.2945	-0.3157	+0.3401

From Table VIII, which gives the lag coefficients concerned, we may infer that —

(i) Rainfall has a uniformly significant negative association, M<sub>2</sub> and M<sub>3</sub> forming exceptions to the rule.

(ii) Relative humidity also has a uniformly significant negative association, M<sub>2</sub> and M<sub>3</sub> again forming exceptions.

(iii) Temperature has a uniformly significant negative association, the coefficients for U P and M<sub>2</sub> alone being insignificant.

(iv) Pressure has a more or less uniform significant positive association all over India, except in U P where it approaches zero.

These zero order coefficients of correlation show how very different are the relationships of the climatic factors with cholera and smallpox respectively. Whereas cholera requires a special atmosphere, the effect of which varies in

M, S-H <sub>0</sub>		NUMBER OF DEATHS FROM SMALLPOX														Total			
		100—200	200—300	300—400	400—500	500—600	600—700	700—800	800—900	900—1,000	1,000—1,100	1,100—1,200	1,200—1,300	1,300—1,400	1,400—1,500		1,500—1,600	1,600—1,700	1,900—2,000
RELATIVE HUMIDITY IN PERCENTAGES	54—57	1		2	1					1									1
	57—60	2		4	1		1		2		1								5
	60—63	4	5	7	1	2			2		1		1						19
	63—66	5	5	7	3	2	2		1		1			1					23
	66—69		3	6	1	2			2										26
	69—72		6	3	7	3	2							1					17
	72—75	1	6	3	7			2	2	2	1	1					1		28
	75—78	3	6	9	7	4	2	2	1		1								35
	78—81	2	5	4	7	6	5	3	1	2			2		1				39
	81—84	7	5	7	12	8	3		2	1	1	1	1			1		1	53
84—87	3	2	9	12	6	3	3	3	1	1	3			1				46	
87—90	2	2	9	3	6	2	2	2	2	1			1					32	
90—93																	.	1	
Total		30	41	67	61	40	20	11	12	17	6	8	5	2	2	1	1	1	325

*Smallpox and rainfall* — Only four coefficients of this group are significant, all being positive —

A	B <sub>3</sub>	C P	M <sub>2</sub>
+0 1707	+0 1574	+0 2153	+0 1636

Increase of rainfall is, therefore, associated with an increase in virulence of the disease in those areas

*Smallpox and relative humidity* — Every coefficient of this group is significant, except that of M<sub>3</sub> which is zero. The fact that both zero order and third order coefficients are uniform in sign and significance is very striking, and makes it possible to infer that relative humidity has a uniform negative association with the virulence of smallpox practically throughout India

*Smallpox and temperature* — The only significant coefficient is that for Assam. It follows that variations of temperature have little association with variations in the virulence of smallpox

*Smallpox and pressure* — Only in

B & O	U P	and M <sub>2</sub>
—0 2443	—0 1803	+0 3125,

are the coefficients significant, and only in M<sub>2</sub> is the association positive

It is to be noted that all four third order coefficients for M<sub>3</sub> are insignificant. In this area, therefore, the virulence of smallpox is independent of climatic factors

It has already been seen that a uniform lag of about two months exists in the association of all four climatic factors with smallpox. Partial correlations were, therefore, computed from lag<sub>2</sub> coefficients of the zero order in order to find out whether the uniform significance of the humidity coefficients was appreciably altered. Table XII gives these third order coefficients, and for purposes of comparison, the corresponding partial coefficients derived from lag<sub>0</sub> coefficients are set out side by side

*Smallpox and rainfall* — The lag<sub>2</sub> coefficients are all positive, and are statistically significant in B<sub>3</sub>, B & O, U P, P<sub>F</sub>, C P, M<sub>1</sub> and M<sub>2</sub>

*Smallpox and relative humidity* — The lag<sub>2</sub> coefficients are all negative and significant as before, except for M<sub>3</sub>, and this confirms the conclusion that, of all the climatic factors, relative humidity plays the most important part in lessening the virulence of smallpox. In other words, lack of humidity produces conditions favourable to increased virulence of smallpox

*Smallpox and temperature* — The lag<sub>2</sub> coefficients are all negative, and all but four are significant. This shows that in the long run temperature also has a mitigating influence on the virulence of smallpox

*Smallpox and pressure* — The lag<sub>2</sub> coefficients vary in sign, only those for U P, P<sub>F</sub>, M<sub>1</sub> and M<sub>2</sub> being significant



*Pressure* affects the association of rainfall with smallpox only slightly It does not affect that of humidity, but affects the association of temperature with smallpox

*Southern Districts Group, Madras Presidency (M<sub>2</sub>)*

*Rainfall* does not affect the associations of humidity, temperature or pressure

*Humidity* does not affect the association of rainfall, but affects those of temperature and pressure to some extent when rainfall is constant

*Temperature* does not affect the associations of rainfall or humidity It affects that of pressure

*Pressure* affects the associations of rainfall and temperature It affects that of humidity greatly

*Central Districts Group, Madras Presidency (M<sub>3</sub>)*

*Rainfall* does not affect the associations of humidity, temperature or pressure

*Humidity* does not affect the association of rainfall, but affects those of temperature and pressure to some extent when rainfall is constant

*Temperature* does not affect the association of rainfall, but affects that of humidity when pressure is not constant It affects that of pressure

*Pressure* does not affect the association of rainfall, but affects that of humidity when temperature is not constant It affects that of temperature

*Bombay Presidency (B<sub>r</sub>)*

*Rainfall* does not affect the associations of humidity or temperature, but affects that of pressure

*Humidity* affects the association of rainfall and also those of temperature and pressure to some extent

*Temperature* does not affect the associations of rainfall, humidity or pressure

*Pressure* affects the associations of rainfall and temperature slightly, but not that of humidity

*Punjab and North-Western Frontier Province (P<sub>r</sub>)*

*Rainfall* has no effect on the associations of humidity, temperature or pressure

*Humidity* affects the association of rainfall slightly, but not those of temperature or pressure

*Temperature* does not affect the associations of rainfall, humidity or pressure

*Pressure* has no effect on the associations of rainfall, humidity or temperature



C P S—H <sub>0</sub>		NUMBER OF DEATHS FROM SMALLPOX																
		0—100	100—200	200—300	300—400	400—500	500—600	600—700	700—800	800—900	900—1,000	1,000—1,100	1,100—1,200	1,200—1,300	1,300—1,400	1,400—1,500	1,500—1,600	Total
RELATIVE HUMIDITY IN PERCENTAGES		20—25	25—30	30—35	35—40	40—45	45—50	50—55	55—60	60—65	65—70	70—75	75—80	80—85	85—90	90—95		
		4		5	1	3			1								1	1
		2		3	1		2			1	4						2	
		1	2	7			4		1	1	1				1		1	
		1	4	4	1		1		4	2	1				1		1	
		6	5	2	2		1			1	1							
		4	9	3			2			1	2				1			
		9	7	4	3		4			1	2				1			
		17	5	2	6		1			1	2							
		14	10	4	1		2			1	1				1			
		4	4	1	1		2			1	1							
		8	6	2	1													
		11	7	6	3		1				2				1			
		9	8	5	1		3				1					1		
				1														1
Total.		90	67	49	21	25	9	19	5	8	6	5	2	4	4	4	1	319

*Pressure* affects the association of rainfall considerably It has no effect on that of humidity, but affects that of temperature

*Rajshahi Division, Bengal (B<sub>1</sub>)*

*Rainfall* has no effect on the associations of humidity, temperature or pressure

*Humidity* affects the association of rainfall, but not those of temperature or pressure

*Temperature* does not affect the associations of rainfall, humidity or pressure

*Pressure* affects the associations of rainfall and temperature, but not that of humidity

*Presidency Division, Bengal (B<sub>2</sub>)*

*Rainfall* does not affect the associations of humidity, temperature or pressure

*Humidity* affects the association of rainfall, but not those of temperature or pressure

*Temperature* does not affect the associations of rainfall, humidity or pressure

*Pressure* does not affect the associations of rainfall, humidity or temperature

*Dacca and Chittagong Division, Bengal (B<sub>3</sub>)*

*Rainfall* does not affect any of the associations of humidity, temperature or pressure

*Humidity* affects the association of rainfall, but not those of temperature or pressure

*Temperature* does not affect any of the associations of rainfall, humidity or pressure

*Pressure* does not affect the associations of rainfall, humidity or temperature

*Burdwan Division, Bengal (B<sub>4</sub>)*

*Rainfall* does not affect any of the associations of humidity, temperature or pressure

*Humidity* affects the association of rainfall, but not those of temperature or pressure

*Temperature* does not affect any of the associations of rainfall, humidity or pressure

*Pressure* does not affect any of the associations of rainfall, humidity or temperature



GENERAL RELATIONSHIP OF THE CLIMATIC FACTORS WITH SMALLPOX  
Table XIV (see Appendix)

*Rainfall*—Rainfall has generally positive coefficients in A, B<sub>3</sub> and C P. In these areas, it has already been shown that relative humidity is not much affected by rainfall. This, although clear as regards A and B<sub>3</sub>, is somewhat difficult of explanation in the case of C P. It may be due to the fact that rainfall is often scanty in this area.

Rainfall coefficients are zero in B<sub>2</sub>, B<sub>4</sub>, P<sub>F</sub> and M<sub>3</sub>, negative in B & O, B<sub>1</sub>, U P, B<sub>V</sub> and M<sub>1</sub>, and varying in M<sub>2</sub>.

*Relative humidity*—All the coefficients for this factor are significant and negative in every area, except in M<sub>2</sub> and M<sub>3</sub>, where they are either slightly negative or zero. The association of humidity with smallpox, therefore, may be said to be uniform.

*Temperature*—The general association of temperature with smallpox is *nil* in B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, P<sub>F</sub>, B<sub>V</sub> and M<sub>1</sub>, negative in A and M<sub>3</sub>, and tends to be positive in B & O, U P, C P and M<sub>2</sub>.

It is possible that, whereas in A and M<sub>3</sub> increase of temperature means a corresponding increase of humidity, in B & O, U P, C P and M<sub>2</sub>, conditions are such that increase of temperature lessens humidity.

*Pressure*—The effect of pressure is significantly positive in M<sub>2</sub>, negative in U P, B & O, A and B<sub>1</sub>, and zero in other areas.

The interpretations of these different methods of analysis of the coefficients may be summarised as follows, the degree of association of each climatic factor in each area being denoted by + and — signs.

Areas	Rainfall	Humidity	Temperature	Pressure
M <sub>1</sub>	—	— —		.
M <sub>2</sub>		—		+ + +
M <sub>3</sub>			—	
B <sub>V</sub>	—	— — —		
P <sub>F</sub>		— —		
U P	—	— —		— —
C. P	+ +	— — —		
B & O	—	— — —	+	— —
A.	+ +	— — —	— —	—
B <sub>1</sub>	—	— —		—
B <sub>2</sub>		— — —		
B <sub>3</sub>	+ +	— — —		
B <sub>4</sub>		— —		



three areas, viz,  $P_r$ ,  $M_3$  and  $B_4$ , smallpox is largely independent of climatic factors, or in other words, these areas are highly endemic with respect to smallpox

#### RELATIVE HUMIDITY versus ABSOLUTE HUMIDITY

Having proved that lack of humidity is directly associated with virulence of smallpox and that this association is only slightly modified by rainfall or pressure in A,  $B_3$ , C P, U P and B & O, it follows that by watching the relative humidity records it should be possible to forecast epidemics of smallpox with some degree of accuracy

Before taking up this question further, however, it seems important to decide whether it is absolute humidity that is involved, as suggested by Sir Leonard Rogers(11), or whether relative humidity is the factor of primary importance as is indicated by the present analysis

The conclusions arrived at by Sir Leonard Rogers are —

(i) 'The smallpox incidence in India is greatest and most uniform from year to year in Madras, the only province not receiving much rain during the south-west monsoon months from June to October'

(ii) 'The greatest smallpox epidemics occur in the low rainfall areas of North-West India and the Central India and Deccan Plateau, while they are least marked in humid Bengal with consistently high rainfall, the epidemics in the former areas nearly invariably follow a greater or less failure of the south-west monsoon rains, accompanied by comparatively low humidity'

(iii) 'The absolute humidity (that is the amount of aqueous vapour as measured by its pressure, a convenient measure of combined humidity and temperature) shows a close relation to smallpox prevalence'

(iv) 'In Madras, both the absolute humidity and smallpox rates are stationary at that season in the absence of the south-west monsoon, while the maximum absolute humidity occurs as early as April and May and is accompanied by the annual moderate early decline of smallpox. The early winter north-east monsoon rains occur during a fall of the mean monthly temperature and of the absolute humidity, thus explaining their failure to prevent the winter increase of smallpox in Madras. The influence of high absolute humidity in checking smallpox thus suffices to explain its seasonal prevalence in all parts of India with their very varying rainfalls and temperatures'

(v) 'It is especially noteworthy that in the North-West Frontier Province alone is the absolute humidity during the south-west monsoon as low as in Madras, as this is the only climatic feature common to these two distant parts of India, and that the smallpox rate is nearly as high'

(vi) 'By watching the absolute humidity in India, and possibly in other tropical continental countries, at the season when they normally average about 0.700 to 0.800, about four-fifths of smallpox epidemics may be foreseen two or three months ahead in time to enable the Public Health authorities to issue

B <sub>4</sub> S—H <sub>0</sub>		NUMBER OF DEATHS FROM SMALLPOX												RELATIVE HUMIDITY IN PERCENTAGES					
		0—200	200—400	400—600	600—800	800—1,000	1,000—1,200	1,200—1,400	1,400—1,600	1,600—1,800	1,800—2,000	2,000—2,200	2,200—2,400	2,400—2,600	2,600—2,800	2,800—3,000	3,000—3,800	4,400—4,600	Total
54—57		1	1	2							1								3
57—60																			2
60—63		1		1			1												3
63—66		2	2		1		1												5
66—69		1	1		2		1												5
69—72		6	3	1	1	1	2		1					1					17
72—75		12	1	1	2	2	1												20
75—78		19	7	4	2	1	1								1				35
78—81		29	7	2	5	2		2	1		1							1	51
81—84		28	12	6	1				1						1		1		50
84—87		27	4	4	6	1				1				1					44
87—90		48	9	7		2			1										67
90—93		9	2																11
TOTAL		183	49	28	20	9	6	2	4	2	1	2		3	2	1	1	1	313

TABLE XV  
Average Absolute Humidity for the period 1896 to 1920

Provinces	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec.
M <sub>1</sub>	0 555	0 613	0 682	0 792	0 846	0 827	0 815	0 815	0 831	0 771	0 645	0 541
M <sub>2</sub>	0 685	0 715	0 767	0 854	0 846	0 790	0 766	0 773	0 785	0 806	0 773	0 694
M <sub>3</sub>	0 681	0 728	0 789	0 882	0 850	0 784	0 789	0 800	0 832	0 849	0 785	0 693
B <sub>Y</sub>	0 360	0 384	0 477	0 597	0 723	0 812	0 812	0 788	0 762	0 643	0 467	0 367
P <sub>Y</sub>	0 252	0 269	0 344	0 412	0 497	0 676	0 851	0 872	0 725	0 448	0 304	0 235
U P	0 301	0 318	0 360	0 429	0 585	0 789	0 913	0 918	0 835	0 588	0 395	0 301
C P	0 306	0 307	0 327	0 389	0 514	0 725	0 806	0 796	0 769	0 558	0 384	0 309
B & O	0 386	0 431	0 498	0 616	0 769	0 885	0 924	0 926	0 898	0 744	0 513	0 381
A.	0 411	0 453	0 545	0 674	0 809	0 917	0 952	0 950	0 921	0 809	0 597	0 449
B <sub>1</sub>	0 387	0 415	0 505	0 684	0 810	0 923	0 966	0 965	0 937	0 807	0 573	0 419
B <sub>2</sub>	0 462	0 548	0 718	0 871	0 943	0 985	0 984	0 980	0 969	0 873	0 633	0 457
B <sub>3</sub>	0 449	0 507	0 666	0 818	0 893	0 947	0 956	0 953	0 947	0 870	0 657	0 485
B <sub>4</sub>	0 392	0 439	0 551	0 754	0 874	0 950	0 973	0 969	0 958	0 827	0 572	0 402



TABLE X—*contd* $B_1$ 

$r_{12} = +0163$	$r_{123} = +1643$ $r_{124} = -0930$ $r_{125} = -1637$	$r_{1234} = +0740$ $r_{1235} = -0073$ $r_{1243} = +0740$ $r_{1245} = -1646$ $r_{1253} = -0073$ $r_{1254} = -1646$	$r_{12345} = -0041$
$r_{13} = -2351$	$r_{132} = -2838$ $r_{134} = -2544$ $r_{135} = -2766$	$r_{1324} = -2486$ $r_{1325} = -2271$ $r_{1342} = -2486$ $r_{1345} = -2821$ $r_{1352} = -2271$ $r_{1354} = -2821$	$r_{13245} = -2324$
$r_{14} = +1208$	$r_{142} = +1512$ $r_{143} = +1564$ $r_{145} = -0176$	$r_{1423} = +0538$ $r_{1425} = -0246$ $r_{1432} = +0538$ $r_{1435} = -0607$ $r_{1452} = -0246$ $r_{1453} = -0607$	$r_{14235} = -0609$
$r_{15} = -1457$	$r_{152} = -2172$ $r_{153} = -2078$ $r_{154} = -0839$	$r_{1523} = -1296$ $r_{1524} = -1597$ $r_{1532} = -1296$ $r_{1534} = -1509$ $r_{1542} = -1597$ $r_{1543} = -1509$	$r_{15234} = -1326$

TABLE XVI  
Average Relative Humidity for the period 1896 to 1920

Provinces	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec.
M <sub>1</sub>	76	71	68	68	67	71	76	78	79	76	74	74
M <sub>2</sub>	80	78	74	73	71	69	71	73	75	80	81	80
M <sub>3</sub>	86	84	85	77	64	62	69	71	77	81	85	85
B <sub>Y</sub>	61	58	56	57	64	75	81	83	81	69	60	59
P <sub>F</sub>	76	71	61	48	40	49	68	74	68	58	63	72
U P	78	69	53	42	46	62	80	84	79	67	68	74
C P	62	53	40	34	39	64	82	86	81	63	59	61
B & O	78	72	60	58	66	78	85	87	85	78	75	76
A	93	84	82	84	86	90	91	91	91	90	90	93
B <sub>1</sub>	88	82	73	75	81	88	90	90	89	86	84	86
B <sub>2</sub>	85	83	80	80	81	85	88	88	86	84	82	82
B <sub>3</sub>	87	83	81	82	82	88	89	89	89	86	85	87
B <sub>4</sub>	81	76	69	73	79	85	88	88	88	83	79	79

TABLE X—*contd* $B_3$ 

$r_{12} = -0128$	$r_{12}^*3 = +2000$ $r_{12}^*4 = -0497$ $r_{12}^*5 = -0482$	$r_{12}^*34 = +1981$ $r_{12}^*35 = +1561$ $r_{12}^*43 = +1981$ $r_{12}^*45 = -0435$ $r_{12}^*53 = +1561$ $r_{12}^*54 = -0435$	$r_{12}^*345 = +1574$
$r_{13} = -3290$	$r_{13}^*2 = -3792$ $r_{13}^*4 = -3372$ $r_{13}^*5 = -3529$	$r_{13}^*24 = -3826$ $r_{13}^*25 = -3794$ $r_{13}^*42 = -3826$ $r_{13}^*45 = -3554$ $r_{13}^*52 = -3794$ $r_{13}^*54 = -3554$	$r_{13}^*245 = -3825$
$r_{14} = +0361$	$r_{14}^*2 = +0601$ $r_{14}^*3 = +0858$ $r_{14}^*5 = +0412$	$r_{14}^*23 = -0813$ $r_{14}^*25 = +0357$ $r_{14}^*32 = -0813$ $r_{14}^*35 = -0604$ $r_{14}^*52 = +0357$ $r_{14}^*53 = -0604$	$r_{14}^*235 = -0638$
$r_{15} = -0168$	$r_{15}^*2 = -0493$ $r_{15}^*3 = -1362$ $r_{15}^*4 = +0260$	$r_{15}^*23 = +0508$ $r_{15}^*24 = -0097$ $r_{15}^*32 = +0508$ $r_{15}^*34 = -1219$ $r_{15}^*42 = -0097$ $r_{15}^*43 = -1219$	$r_{15}^*234 = -0062$

- (18) GREIG, E D W (1913-14)      An investigation of Cholera Convalescents and Con-  
tacts in India *Ind Jour Med Res*, 1
- (19) GREIG, E D W (1913-14)      An investigation of the occurrence of the comma  
bacillus in the urine in cases of cholera *Ind Jour  
Med Res*, 1 .
- (20) JOHN C McVAIL (1907)      The Prevention of Infectious Diseases
- (21) T F RICKETS and J B      Diagnosis of Smallpox  
BYLES (1908)
- (22) E J EDWARDS (1902)      Smallpox and Vaccination in Europe
- (23) J D ROLLESTON (1925)      Acute Infectious Diseases

TABLE X—*contd*

B. &amp; O.

$r_{12} = -1302$	$r_{123} = +2373$ $r_{124} = -2979$ $r_{125} = -4149$	$r_{1234} = +0972$ $r_{1235} = -0632$ $r_{1243} = +0972$ $r_{1245} = -4072$ $r_{1253} = -0632$ $r_{1254} = -4072$	$r_{12345} = -0507$
$r_{13} = -4225$	$r_{132} = -4597$ $r_{134} = -4353$ $r_{135} = -4925$	$r_{1324} = -3449$ $r_{1325} = -2977$ $r_{1342} = -3449$ $r_{1345} = -4920$ $r_{1352} = -2977$ $r_{1354} = -4920$	$r_{13245} = -3063$
$r_{14} = +2069$	$r_{142} = +3357$ $r_{143} = +2359$ $r_{145} = +0915$	$r_{1423} = +0937$ $r_{1425} = +0255$ $r_{1432} = +0937$ $r_{1435} = -0879$ $r_{1452} = +0255$ $r_{1453} = -0879$	$r_{14235} = -0796$
$r_{15} = -1895$	$r_{152} = -4338$ $r_{153} = -3333$ $r_{154} = -0354$	$r_{1523} = -2489$ $r_{1524} = -2927$ $r_{1532} = -2489$ $r_{1534} = -2569$ $r_{1542} = -2927$ $r_{1543} = -2569$	$r_{15234} = -2443$

## NUMBER OF DEATHS FROM SMALLPOX

 $M_2 S-H_0$ 

RELATIVE HUMIDITY IN PERCENTAGES

RELATIVE HUMIDITY IN PERCENTAGES	NUMBER OF DEATHS FROM SMALLPOX																								
	17	34	43	36	36	46	31	13	17	16	5	6	4	4	3	3	3	1	1	2	1	1	2	2	
64—66	1				1																				
66—68		2	2		2		1																		
68—70	1	6	4	3	2	3	4		1	1	2	1							1				1		
70—72	2	7	3	5	2	4	5	2	2	2	1	2		1		1	2								
72—74	1	4	5	8	7	8	3	3	4	4	1	1													
74—76	3	6	7	4	6	3	4	1	2	1			3			2					1				
76—78	1	1	5	4	5	8	3	2	3			2		1											
78—80	3	2	6	5	3	9	7	1	2	3	1			1					1						
80—82	3	4	9	2	5	5	2			2				1	2			1							
82—84	1	1	2	4	1	5	2	3	2	1			1												
84—86	1			1	2				1	2															
86—88						1																			
88—90		1																							
Total	17	34	43	36	36	46	31	13	17	16	5	6	4	4	3	3	3	1	1	2	1	1	2	325	

TABLE X—contd

C. P

$r_{12} = -0.770$	$r_{12.3} = +2966$ $r_{12.4} = -1554$ $r_{12.5} = -1809$	$r_{12.34} = +2149$ $r_{12.35} = +2143$ $r_{12.43} = +2149$ $r_{12.45} = -0570$ $r_{12.53} = +2143$ $r_{12.54} = -0570$	$r_{12.345} = +2153$
$r_{13} = -3371$	$r_{13.2} = -4321$ $r_{13.4} = -3145$ $r_{13.5} = -3844$	$r_{13.24} = -3454$ $r_{13.25} = -3990$ $r_{13.42} = -3454$ $r_{13.45} = -2813$ $r_{13.52} = -3990$ $r_{13.54} = -2813$	$r_{13.245} = -3450$
$r_{14} = +2480$	$r_{14.2} = +2805$ $r_{14.3} = +2142$ $r_{14.5} = +2817$	$r_{14.23} = +0480$ $r_{14.25} = +2264$ $r_{14.32} = +0480$ $r_{14.35} = +0728$ $r_{14.52} = +2264$ $r_{14.53} = +0728$	$r_{14.235} = +0769$
$r_{15} = -0.773$	$r_{15.2} = -1811$ $r_{15.3} = -2100$ $r_{15.4} = +1577$	$r_{15.23} = +0074$ $r_{15.24} = +0632$ $r_{15.32} = +0074$ $r_{15.34} = -0588$ $r_{15.42} = +0632$ $r_{15.43} = -0588$	$r_{15.234} = +0607$

RELATIVE HUMIDITY IN PERCENTAGES	B <sub>v</sub> S—H <sub>0</sub>	NUMBER OF DEATHS FROM SMALLPOX												Total									
		0—200	200—400	400—600	600—800	800—1,000	1,000—1,200	1,200—1,400	1,400—1,600	1,600—1,800	1,800—2,000	2,000—2,200	2,200—2,400	2,400—2,600	2,600—2,800	2,800—3,000	3,000—3,200	3,200—3,400	3,400—3,600	3,600—3,800	3,800—4,000	Total	
	50—53	2	2	2	1	2	2														1		12
	53—56	6	4	5	2				1	1	1												20
	56—59	8	13	7	3	4	3	1									1				1		41
	59—62	12	6	7	5	3	2		1														37
	62—65	11	8	2	4				1							1							27
	65—68	9	6	4		1																	20
	68—71	7					1																8
	71—74	7	2	3	1																		13
	74—77	5	5	1																			11
	77—80	12	6																				18
	80—83	21	7	2																			30
	83—86	18	2																				20
	86—89	2																					2
	TOTAL	120	61	33	16	10	8	1	2	1	1	2	1				1						259



TABLE X—*contd* $B_v$ 

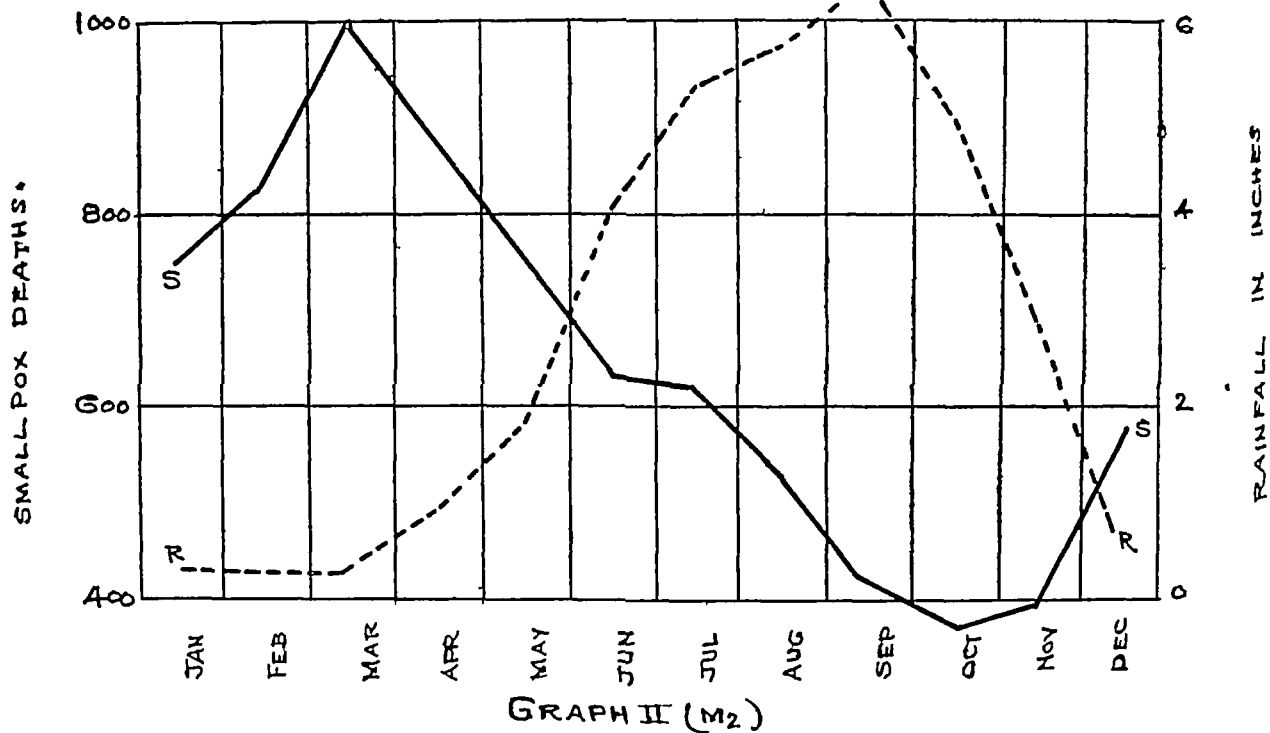
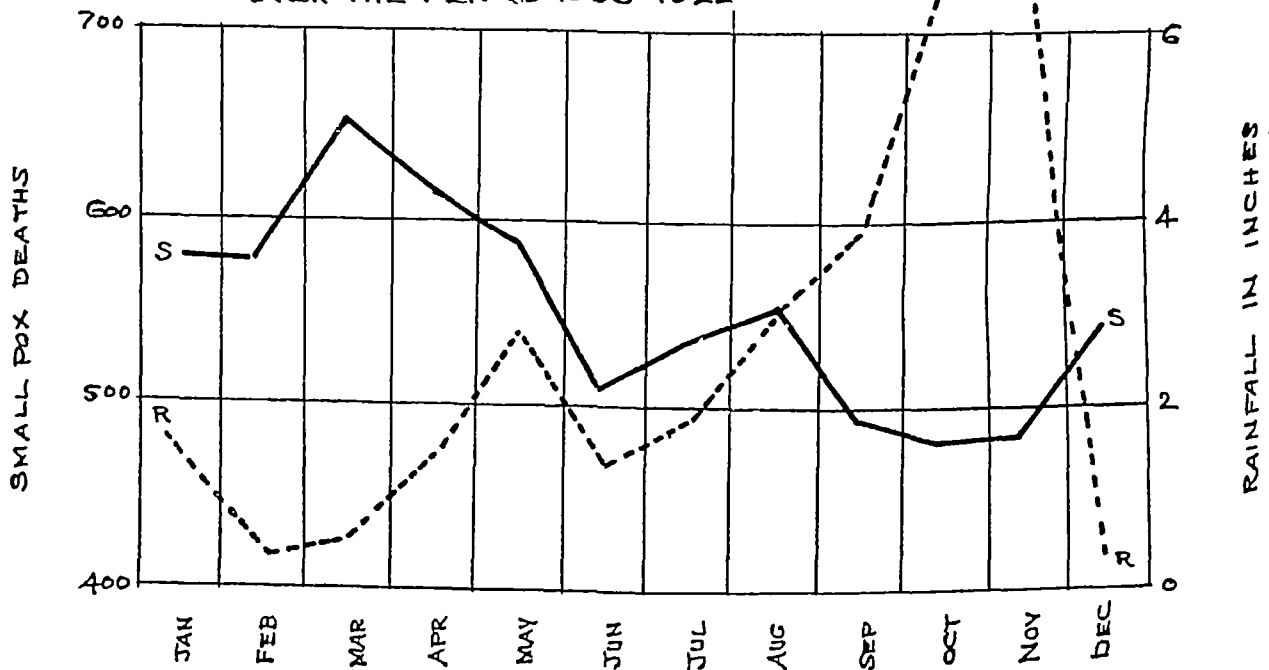
$r_{12} = -2480$	$r_{12}^{\cdot 3} = +0950$ $r_{12}^{\cdot 4} = -2343$ $r_{12}^{\cdot 5} = -1399$	$r_{12}^{\cdot 34} = +0839$ $r_{12}^{\cdot 35} = +0577$ $r_{12}^{\cdot 43} = +0839$ $r_{12}^{\cdot 45} = -1090$ $r_{12}^{\cdot 53} = +0577$ $r_{12}^{\cdot 54} = -1090$	$r_{12}^{\cdot 345} = +0789$
$r_{13} = -3777$	$r_{13}^{\cdot 2} = -3077$ $r_{13}^{\cdot 4} = -3850$ $r_{13}^{\cdot 5} = -3358$	$r_{13}^{\cdot 24} = -3241$ $r_{13}^{\cdot 25} = -3131$ $r_{13}^{\cdot 42} = -3241$ $r_{13}^{\cdot 45} = -3230$ $r_{13}^{\cdot 52} = -3131$ $r_{13}^{\cdot 54} = -3230$	$r_{13}^{\cdot 245} = -3150$
$r_{14} = -0873$	$r_{14}^{\cdot 2} = +0253$ $r_{14}^{\cdot 3} = +1186$ $r_{14}^{\cdot 5} = +1169$	$r_{14}^{\cdot 23} = +1099$ $r_{14}^{\cdot 25} = +0768$ $r_{14}^{\cdot 32} = +1099$ $r_{14}^{\cdot 35} = +0655$ $r_{14}^{\cdot 52} = +0768$ $r_{14}^{\cdot 53} = +0655$	$r_{14}^{\cdot 235} = +0848$
$r_{15} = +2099$	$r_{15}^{\cdot 2} = +0365$ $r_{15}^{\cdot 3} = -1036$ $r_{15}^{\cdot 4} = +2233$	$r_{15}^{\cdot 23} = -0710$ $r_{15}^{\cdot 24} = +0812$ $r_{15}^{\cdot 32} = -0710$ $r_{15}^{\cdot 34} = -0304$ $r_{15}^{\cdot 42} = +0812$ $r_{15}^{\cdot 43} = -0304$	$r_{15}^{\cdot 234} = +0107$

U. P. S—H <sub>0</sub>		NUMBER OF DEATHS FROM SMALLPOX																	Total.	
		0—1,000	1,000—2,000	2,000—3,000	3,000—4,000	4,000—5,000	5,000—6,000	6,000—7,000	7,000—8,000	8,000—9,000	9,000—10,000	10,000—11,000	11,000—12,000	12,000—13,000	13,000—20,000	20,000—21,000	21,000—22,000	Total.		
RELATIVE HUMIDITY IN PERCENTAGES	32—36	4	3															1	8	
	36—40	7			1								1						9	
	40—44	9	4	1	1	1								1					18	
	44—48	15	1	1	1			1											20	
	48—52	9		2	1									1					12	
	52—56	8	3		1						1								13	
	56—60	8	2	2															12	
	60—64	18	2	1		2			1										25	
	64—68	22	4	3						1									30	
	68—72	38	1		2	1													42	
	72—76	23	1																24	
	76—80	38				1													41	
80—84	21	2																24		
84—88	36	3	2															42		
88—92	4		1															5		
TOTAL		280	56	13	7	5	3	1	2	1	1	1	2	1			1	325		

TABLE X—*contd* $M_2$ 

$r_{12} = -1279$	$r_{123} = -1041$ $r_{124} = -1293$ $r_{125} = -1257$	$r_{1234} = -0844$ $r_{1235} = +1425$ $r_{1243} = -0844$ $r_{1245} = -0560$ $r_{1253} = +1425$ $r_{1254} = -0560$	$r_{12345} = +1636$
$r_{13} = -0748$	$r_{132} = -0028$ $r_{134} = -1019$ $r_{135} = -2430$	$r_{1324} = -0274$ $r_{1325} = -2517$ $r_{1342} = -0274$ $r_{1345} = -1875$ $r_{1352} = -2517$ $r_{1354} = -1875$	$r_{13245} = -2408$
$r_{14} = +0066$	$r_{142} = -0197$ $r_{143} = -0696$ $r_{145} = +1971$	$r_{1423} = -0335$ $r_{1425} = +1627$ $r_{1432} = -0335$ $r_{1435} = +1202$ $r_{1452} = +1627$ $r_{1453} = +1202$	$r_{14235} = +1444$
$r_{15} = +1328$	$r_{152} = +1306$ $r_{153} = +2653$ $r_{154} = +2360$	$r_{1523} = +2818$ $r_{1524} = +2067$ $r_{1532} = +2818$ $r_{1534} = +2817$ $r_{1542} = +2067$ $r_{1543} = +2817$	$r_{15234} = +3125$

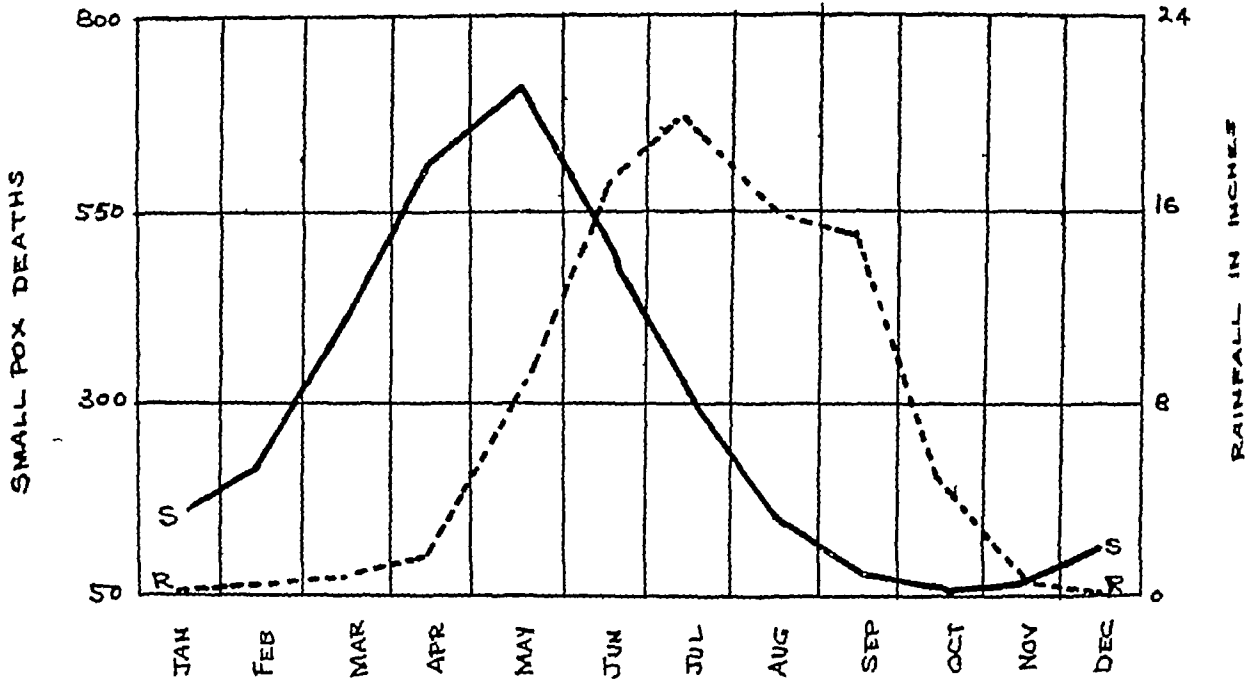
NUMBER OF DEATHS FROM SMALLPOX																			
B & O S—H <sub>0</sub>	RELATIVE HUMIDITY IN PERCENTAGES																		
	0-300	300-600	600-900	900-1,200	1,200-1,500	1,500-1,800	1,800-2,100	2,100-2,400	2,400-2,700	2,700-3,000	3,000-3,300	3,300-3,600	3,600-3,900	3,900-4,200	4,200-4,500	4,500-4,800	4,800-5,100	5,100-5,400.	Total.
45-48	1			1		1							1						1
48-51						1													
51-54		1					1			2				1					
54-57			3	2			2	1		1	2								
57-60		4	2	2	2	1		1	1					1					
60-63		3	2	1	3		2	1		2									
63-66	2	4	2	2	1	2	1		1		2			2		1			
66-69	2	7	4	2	2	1	1	2								1		1	
69-72	3	3	3	1	3	2	1	1		1	1	1			1				
72-75	5	5	1	4	1	1	1	1			1								
75-78	11	13	9	3	4	4	1		1	1						1			
78-81	19	11	3	5	3		1	1											
81-84	13	7	2	3	1	3			1										
84-87	10	14	7	6	2	3		2											
87-90	13	5	6	4			1												
Total	79	77	44	34	22	18	12	10	4	6	6	1	2	4	1	3		2	325

GRAPH I ( $M_1$ ).AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD  
1896-1922AVERAGE MONTHLY MORTALITY FROM SMALLPOX  
OVER THE PERIOD 1896-1922

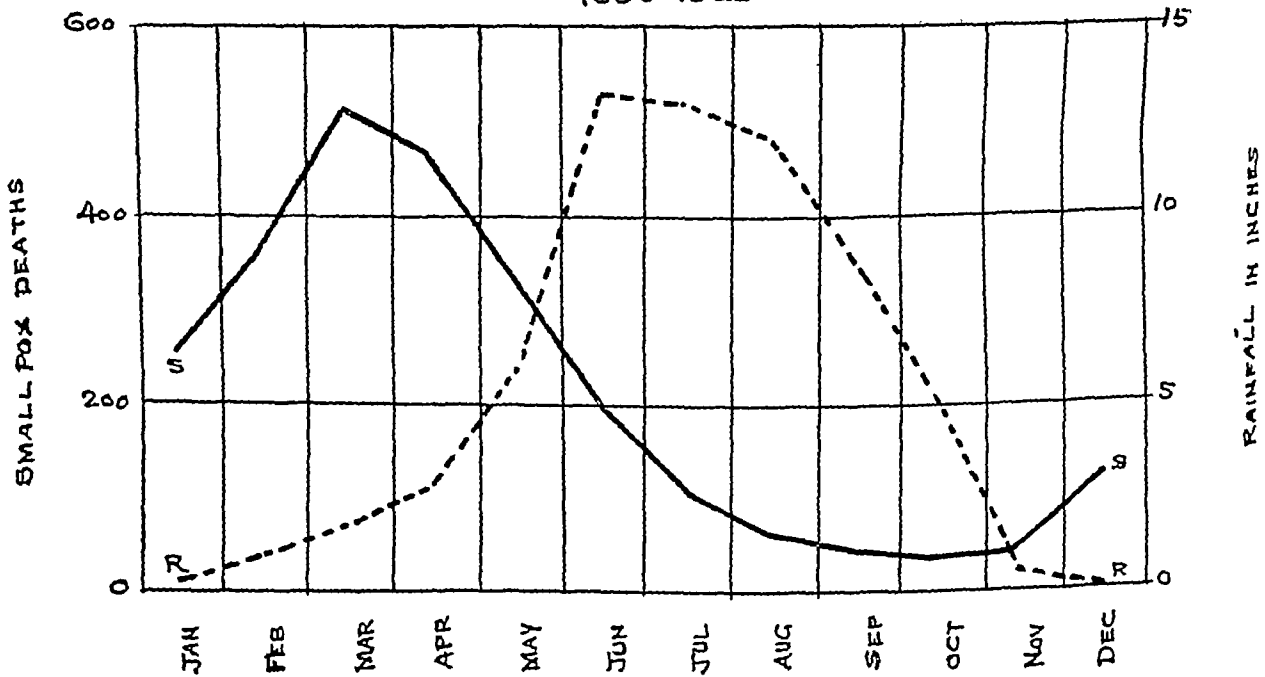
RELATIVE HUMIDITY IN PERCENTAGES	NUMBER OF DEATHS FROM SMALLPOX													Total			
	209	45	17	17	5	5	5	3	2	1	1	2	1		1	1	1
B <sub>1</sub> S—H <sub>0</sub>																	
54—57																	
57—60																	
60—63																	
63—66		1															
66—69	2	1	3														
69—72	4		1	1													
72—75	7	1	2		1												
75—78	6	4	2	2													
78—81	11	6	1	2	2												
81—84	26	7	2	2	2												
84—87	45	5	2	2	2	2											
87—90	74	11	2	5													
90—93	34	9	2	3													
Total	209	45	17	17	5	5	3	2	1	1	1	2	1	1	1	1	313

GRAPH V (B<sub>1</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD  
1896-1922

GRAPH VI (B<sub>2</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD  
1896-1922

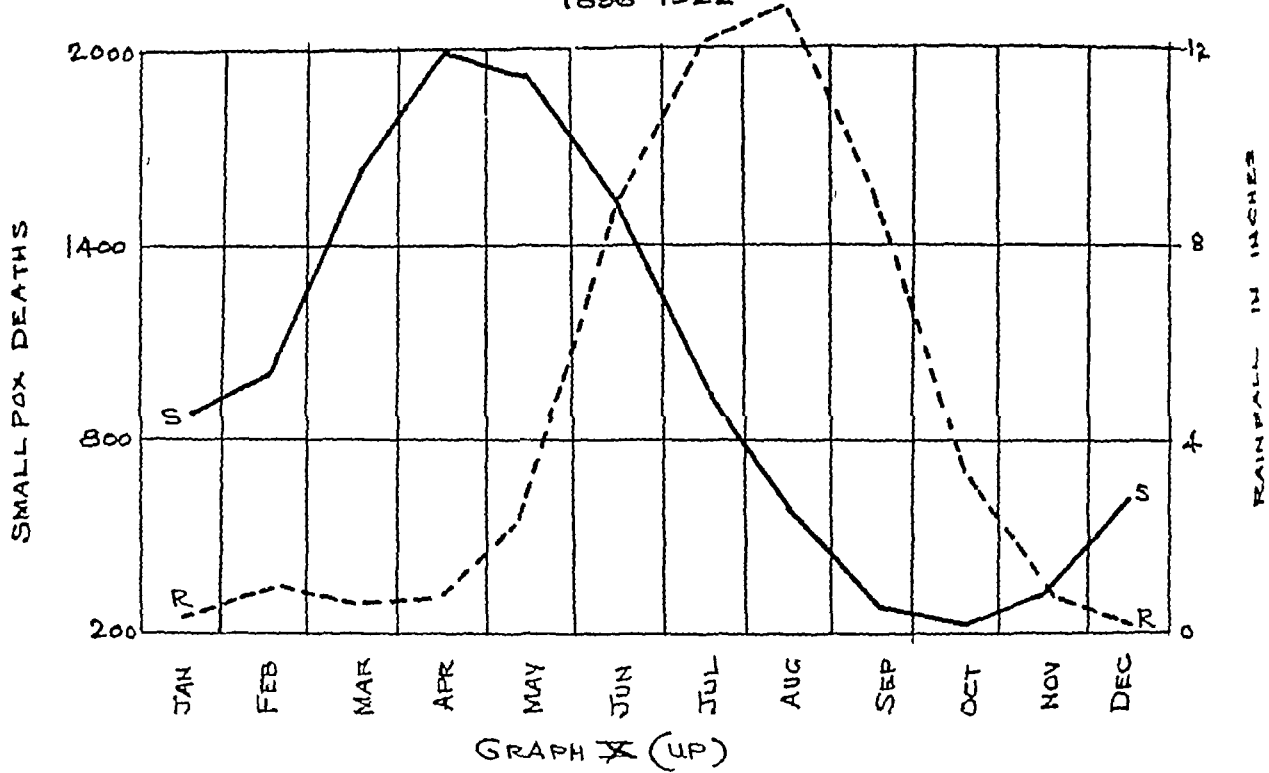


RELATIVE HUMIDITY IN PERCENTAGES	B <sub>3</sub> S-H <sub>0</sub>	NUMBER OF DEATHS FROM SMALLPOX																			Total
		112	56	44	23	21	14	12	9	6	2	3	1	2	2	3	1	1	1	313	
73-74			1																		
74-75																					
77-78																					
78-79																					
79-80																					
80-81																					
81-82																					
82-83																					
83-84																					
84-85																					
85-86																					
86-87																					
87-88																					
88-89																					
89-90																					
90-91																					
91-92																					



GRAPH IX (B20)

AVERAGE MONTHLY MORTALITY FROM SMALL POX OVER THE PERIOD 1896-1922



AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD 1896-1922

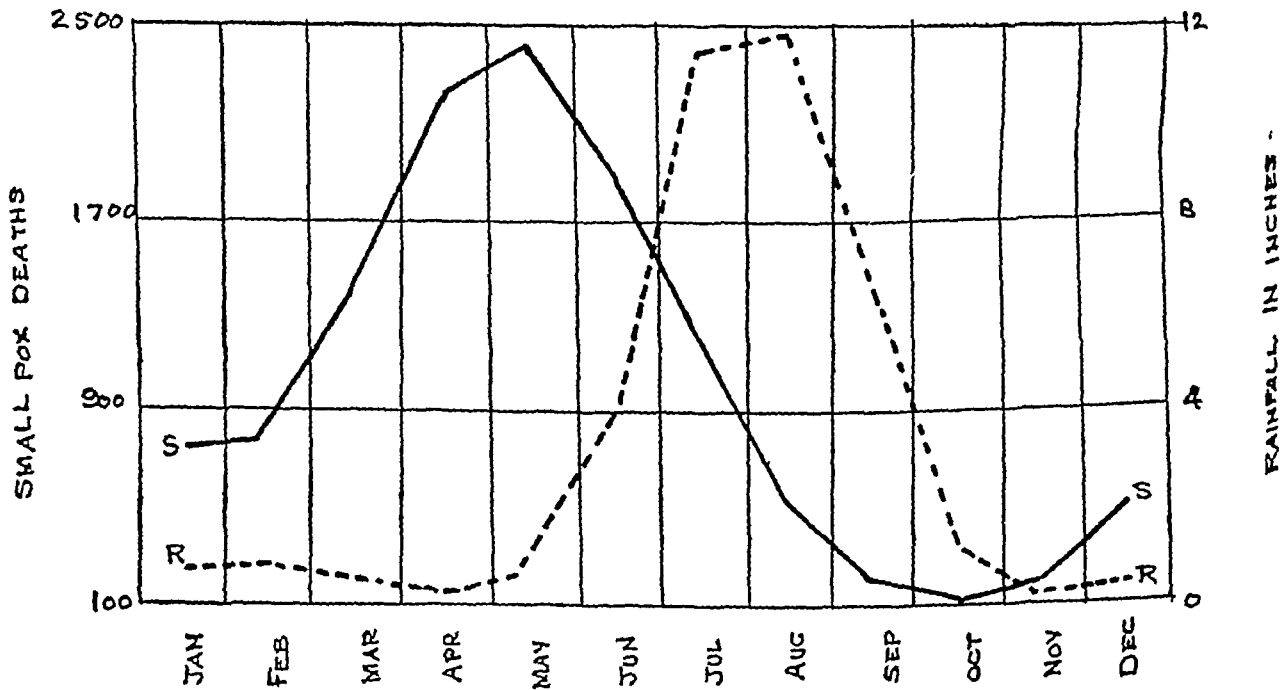


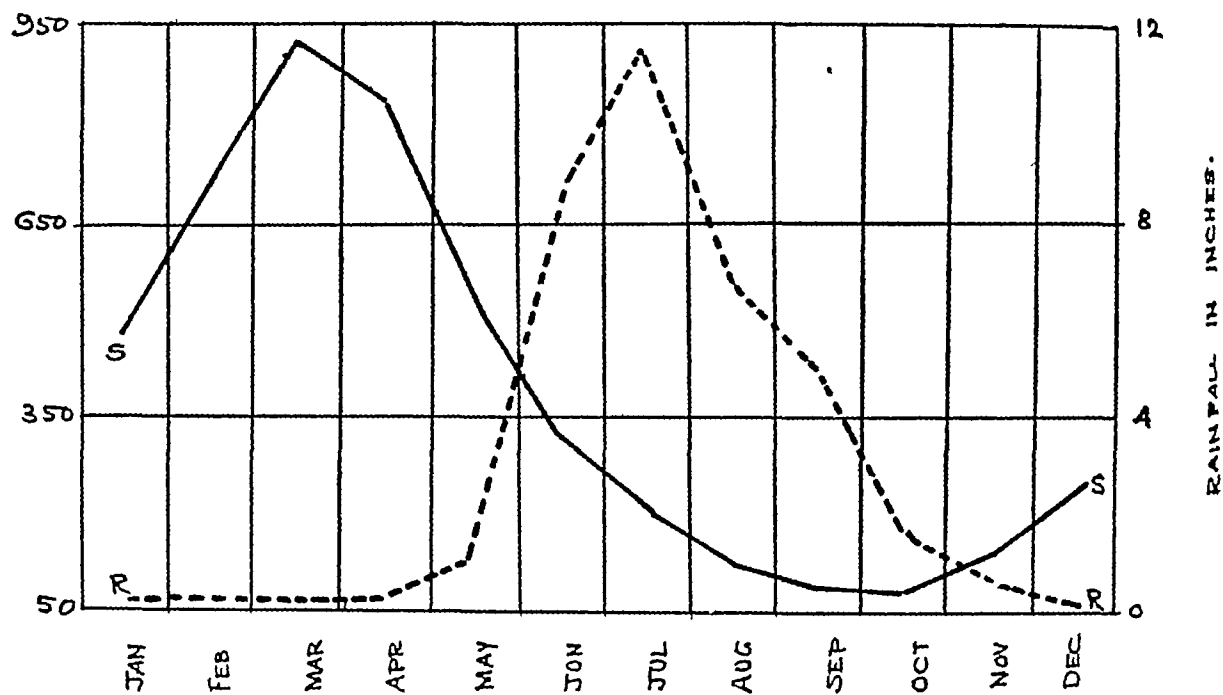
TABLE X

A

$r_{12} = +.1678$	$r_{12}^*3 = +2124$ $r_{12}^*4 = +1716$ $r_{12}^*5 = -0010$	$r_{12}^*34 = +.3138$ $r_{12}^*35 = +1210$ $r_{12}^*43 = +.3138$ $r_{12}^*45 = +.0195$ $r_{12}^*53 = +1210$ $r_{12}^*54 = +0195$	$r_{12}^*345 = +1707$
$r_{13} = -.3143$	$r_{13}^*2 = -3384$ $r_{13}^*4 = -3074$ $r_{13}^*5 = -3083$	$r_{13}^*24 = -3985$ $r_{13}^*25 = -3292$ $r_{13}^*42 = -3985$ $r_{13}^*45 = -.3442$ $r_{13}^*52 = -3292$ $r_{13}^*54 = -3442$	$r_{13}^*245 = -3792$
$r_{14} = +.0829$	$r_{14}^*2 = -0904$ $r_{14}^*3 = +.0461$ $r_{14}^*5 = -1812$	$r_{14}^*23 = -.2404$ $r_{14}^*25 = -1824$ $r_{14}^*32 = -2404$ $r_{14}^*35 = -.2406$ $r_{14}^*52 = -1824$ $r_{14}^*53 = -.2406$	$r_{14}^*235 = -.2677$
$r_{15} = -.1893$	$r_{15}^*2 = -0889$ $r_{15}^*3 = -1785$ $r_{15}^*4 = -2471$	$r_{15}^*23 = +.0313$ $r_{15}^*24 = -1817$ $r_{15}^*32 = +.0313$ $r_{15}^*34 = -.2931$ $r_{15}^*42 = -1817$ $r_{15}^*43 = -.2931$	$r_{15}^*234 = -1251$

## GRAPH XIII (BV)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD  
1896-1922

GRAPH XIV (M<sub>1</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY

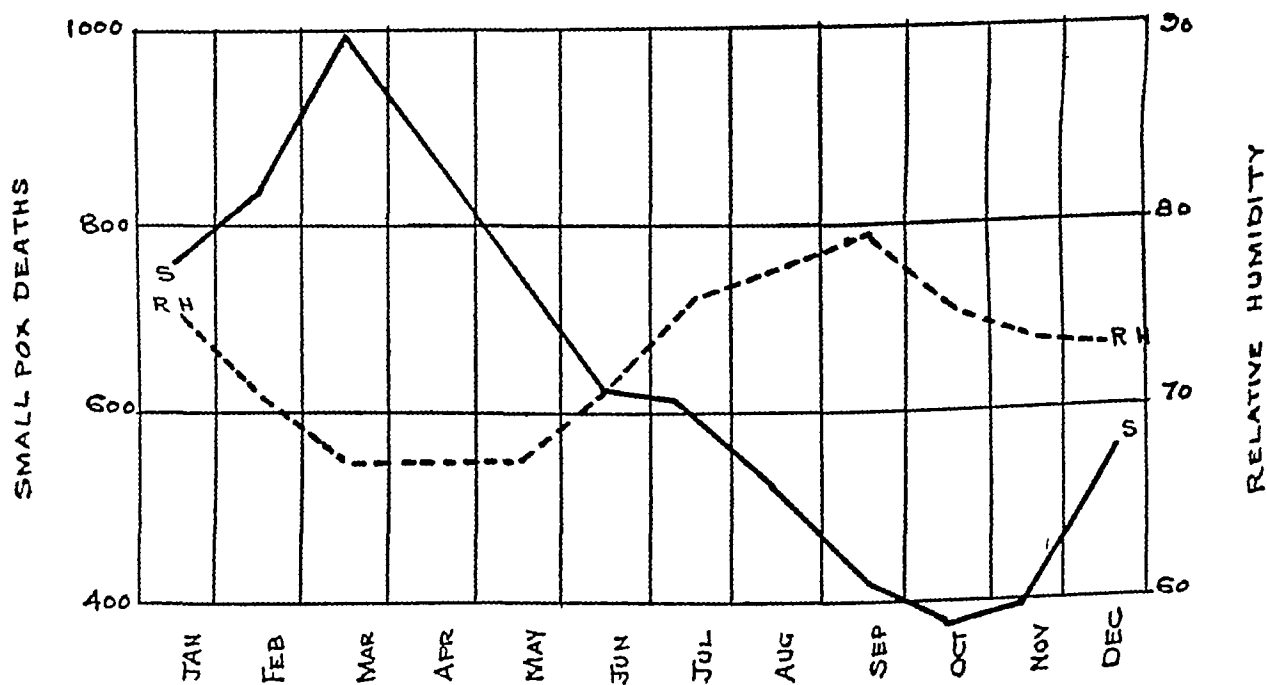
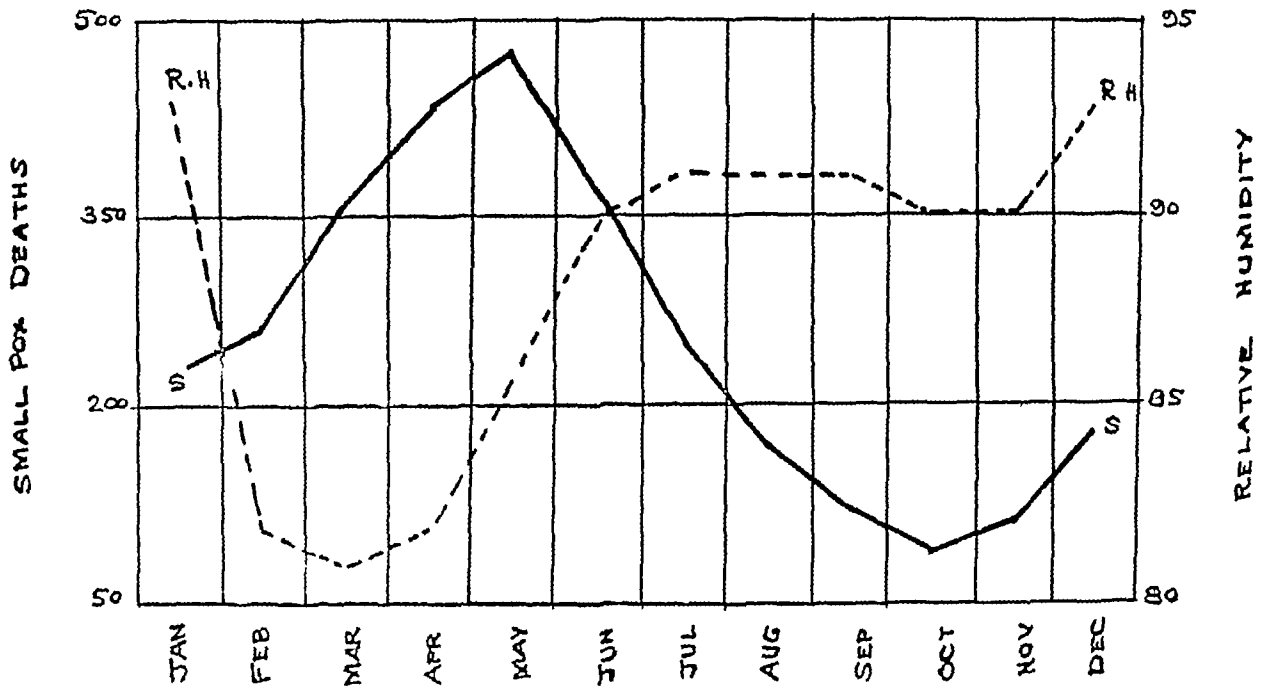


TABLE X—contd  
B<sub>2</sub>

$r_{12} = -0.1550$	$r_{12}^*3 = +0.176$ $r_{12}^*4 = -0.1670$ $r_{12}^*5 = -0.1176$	$r_{12}^*34 = +0.264$ $r_{12}^*35 = +0.374$ $r_{12}^*43 = +0.264$ $r_{12}^*45 = -0.0771$ $r_{12}^*53 = +0.374$ $r_{12}^*54 = -0.0771$	$r_{12}^*345 = +0.399$
$r_{13} = -0.3112$	$r_{13}^*2 = -0.2736$ $r_{13}^*4 = -0.3092$ $r_{13}^*5 = -0.2942$	$r_{13}^*24 = -0.2650$ $r_{13}^*25 = -0.2738$ $r_{13}^*42 = -0.2650$ $r_{13}^*45 = -0.2689$ $r_{13}^*52 = -0.2738$ $r_{13}^*54 = -0.2689$	$r_{13}^*245 = -0.2611$
$r_{14} = -0.0379$	$r_{14}^*2 = +0.0733$ $r_{14}^*3 = -0.0033$ $r_{14}^*5 = +0.1242$	$r_{14}^*23 = -0.0199$ $r_{14}^*25 = +0.0869$ $r_{14}^*32 = -0.0199$ $r_{14}^*35 = +0.0063$ $r_{14}^*52 = +0.0869$ $r_{14}^*53 = +0.0063$	$r_{14}^*235 = +0.0150$
$r_{15} = +0.1064$	$r_{15}^*2 = -0.0316$ $r_{15}^*3 = +0.0066$ $r_{15}^*4 = +0.1587$	$r_{15}^*23 = +0.0337$ $r_{15}^*24 = +0.0562$ $r_{15}^*32 = +0.0337$ $r_{15}^*34 = +0.0085$ $r_{15}^*42 = +0.0562$ $r_{15}^*43 = +0.0085$	$r_{15}^*234 = +0.0311$

GRAPH XVII (A)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY

GRAPH XVIII (B<sub>1</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY

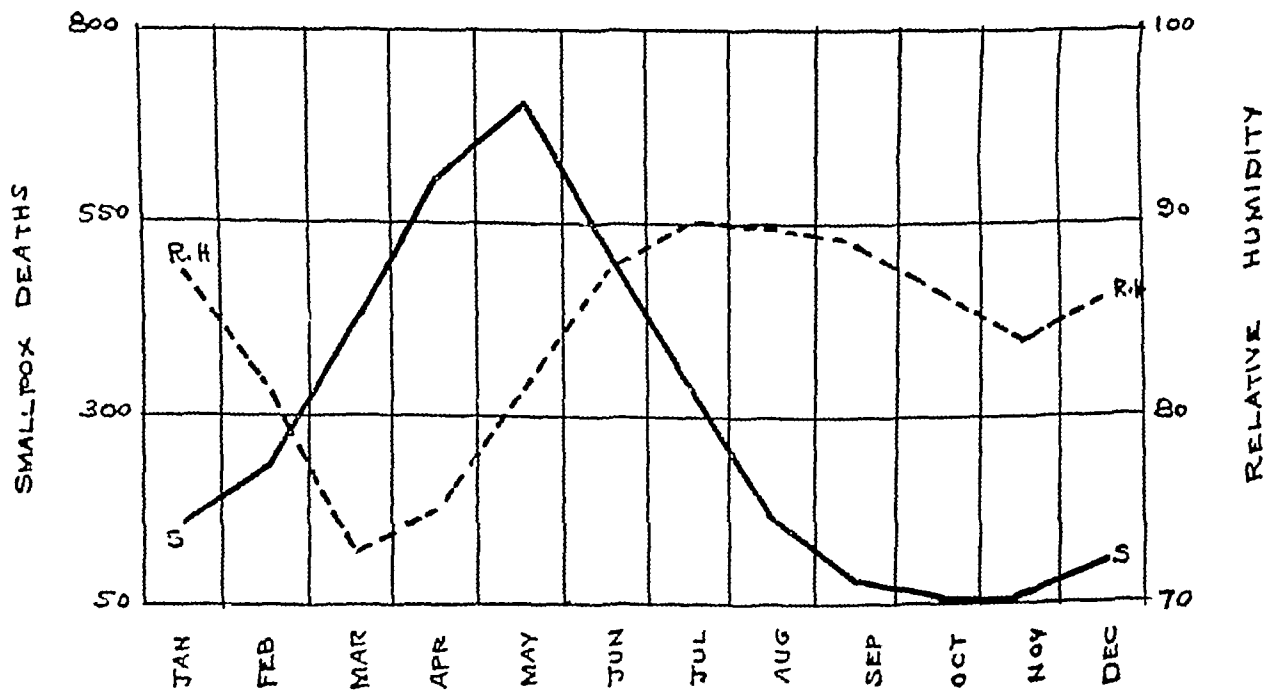
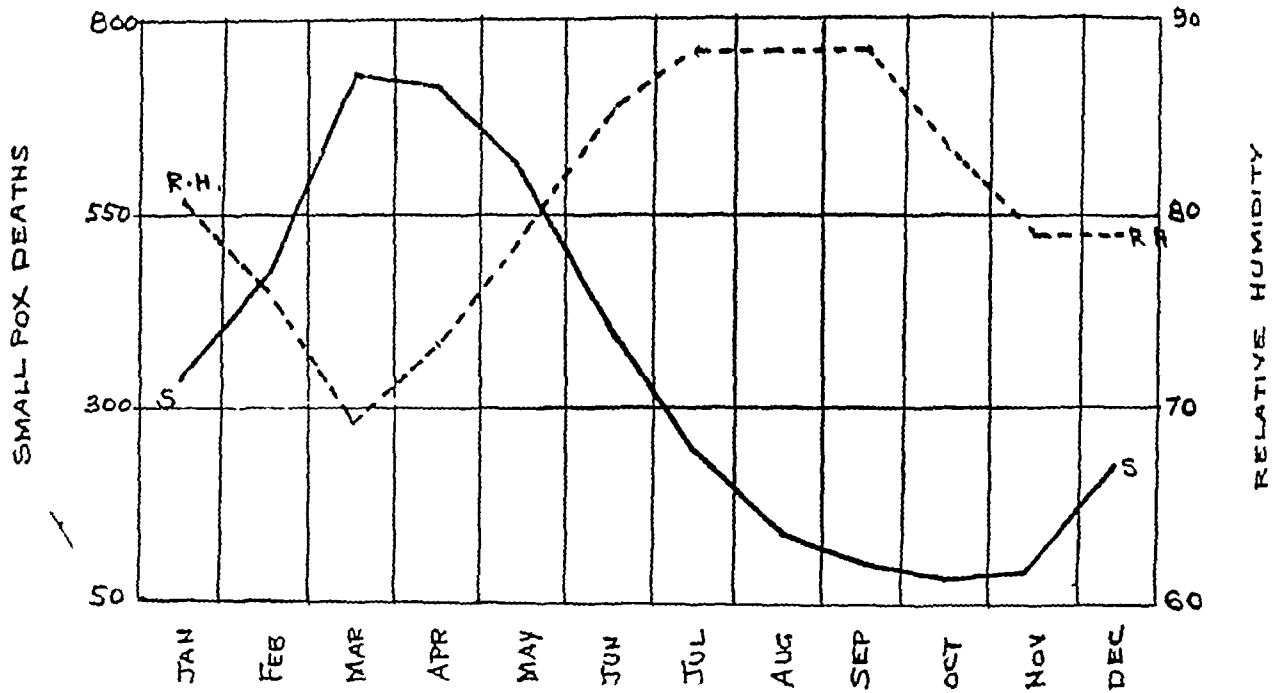


TABLE X—contd

 $B_4$ 

$r_{12} = -0953$	$r_{123} = +0457$ $r_{124} = -1352$ $r_{125} = -1256$	$r_{1234} = -0067$ $r_{1235} = -0181$ $r_{1243} = -0067$ $r_{1245} = -1142$ $r_{1253} = -0181$ $r_{1254} = -1142$	$r_{12345} = -0139$
$r_{13} = -2006$	$r_{132} = -1830$ $r_{134} = -2216$ $r_{135} = -2192$	$r_{1324} = -1774$ $r_{1325} = -1821$ $r_{1342} = -1774$ $r_{1345} = -2102$ $r_{1352} = -1821$ $r_{1354} = -2102$	$r_{13245} = -1781$
$r_{14} = +0225$	$r_{142} = +0988$ $r_{143} = +0986$ $r_{145} = +0749$	$r_{1423} = +0877$ $r_{1425} = +0535$ $r_{1432} = +0877$ $r_{1435} = +0389$ $r_{1452} = +0535$ $r_{1453} = +0389$	$r_{14235} = +0371$
$r_{15} = +0149$	$r_{152} = -0833$ $r_{153} = -0914$ $r_{154} = +0731$	$r_{1523} = -0812$ $r_{1524} = -0050$ $r_{1532} = -0812$ $r_{1534} = -0119$ $r_{1542} = -0050$ $r_{1543} = -0119$	$r_{15234} = -0170$

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY

GRAPH XXII (B<sub>60</sub>).

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY.

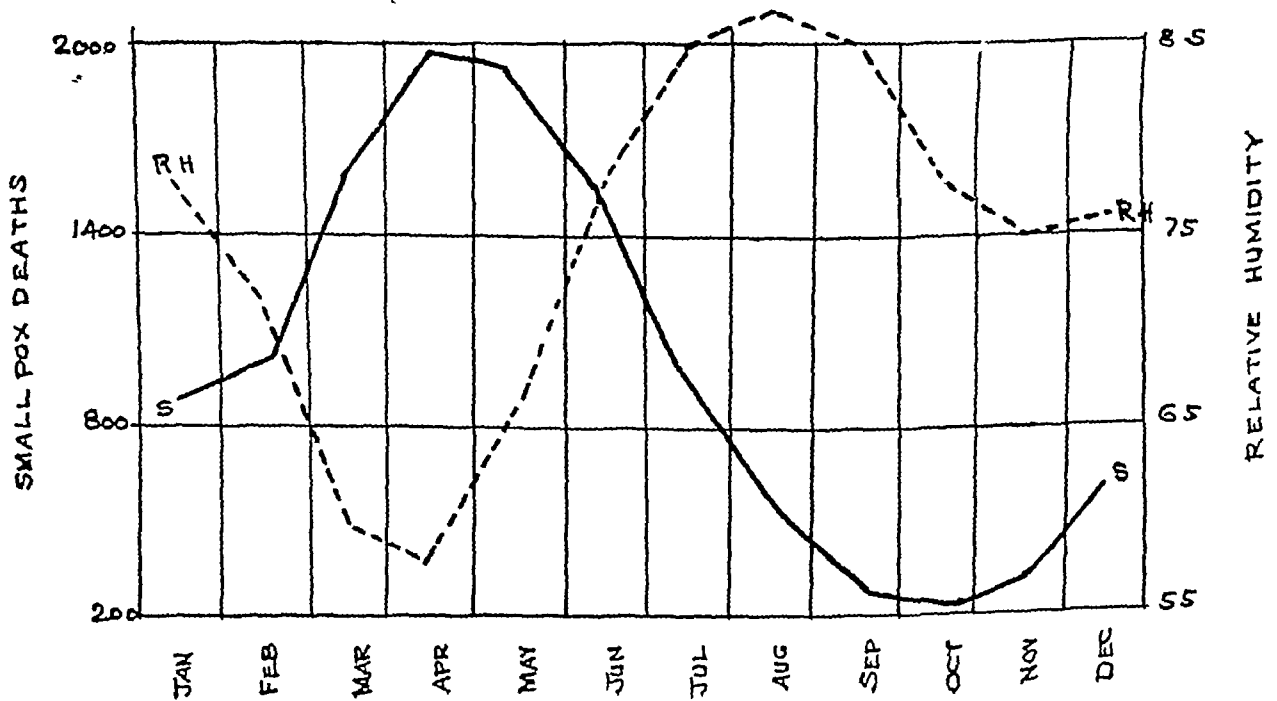


TABLE X—contd

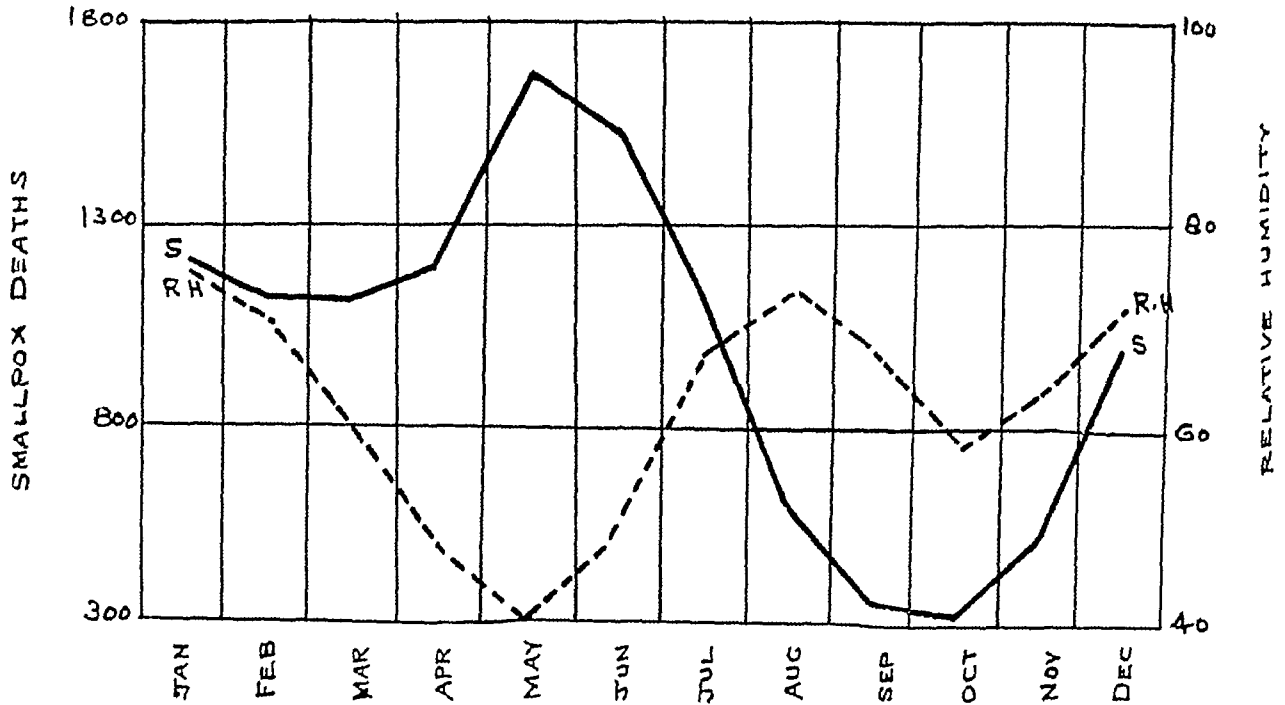
U. P.

$r_{12} = -0524$	$r_{12}^*3 = +1225$ $r_{12}^*4 = -1244$ $r_{12}^*5 = -1902$	$r_{12}^*34 = +0953$ $r_{12}^*35 = -0067$ $r_{12}^*43 = +0953$ $r_{12}^*45 = -1922$ $r_{12}^*53 = -0067$ $r_{12}^*54 = -1922$	$r_{12}^*345 = +0406$
$r_{13} = -2455$	$r_{13}^*2 = -2679$ $r_{13}^*4 = -2181$ $r_{13}^*5 = -2476$	$r_{13}^*24 = -2034$ $r_{13}^*25 = -1615$ $r_{13}^*42 = -2034$ $r_{13}^*45 = -2787$ $r_{13}^*52 = -1615$ $r_{13}^*54 = -2787$	$r_{13}^*245 = -2093$
$r_{14} = +1401$	$r_{14}^*2 = +1792$ $r_{14}^*3 = +0798$ $r_{14}^*5 = +0024$	$r_{14}^*23 = -0195$ $r_{14}^*25 = -0273$ $r_{14}^*32 = -0195$ $r_{14}^*35 = -1320$ $r_{14}^*52 = -0273$ $r_{14}^*53 = -1320$	$r_{14}^*235 = -1376$
$r_{15} = -1671$	$r_{15}^*2 = -2459$ $r_{15}^*3 = -1702$ $r_{15}^*4 = -0920$	$r_{15}^*23 = -1193$ $r_{15}^*24 = -1732$ $r_{15}^*32 = -1193$ $r_{15}^*34 = -1994$ $r_{15}^*42 = -1732$ $r_{15}^*43 = -1994$	$r_{15}^*234 = -1803$



GRAPH XXV (PF)

AVERAGE MONTHLY MORTALITY FROM SMALL POX AND AVERAGE  
RELATIVE HUMIDITY

GRAPH XXVI (BY)

AVERAGE MONTHLY MORTALITY FROM SMALL POX AND AVERAGE  
RELATIVE HUMIDITY

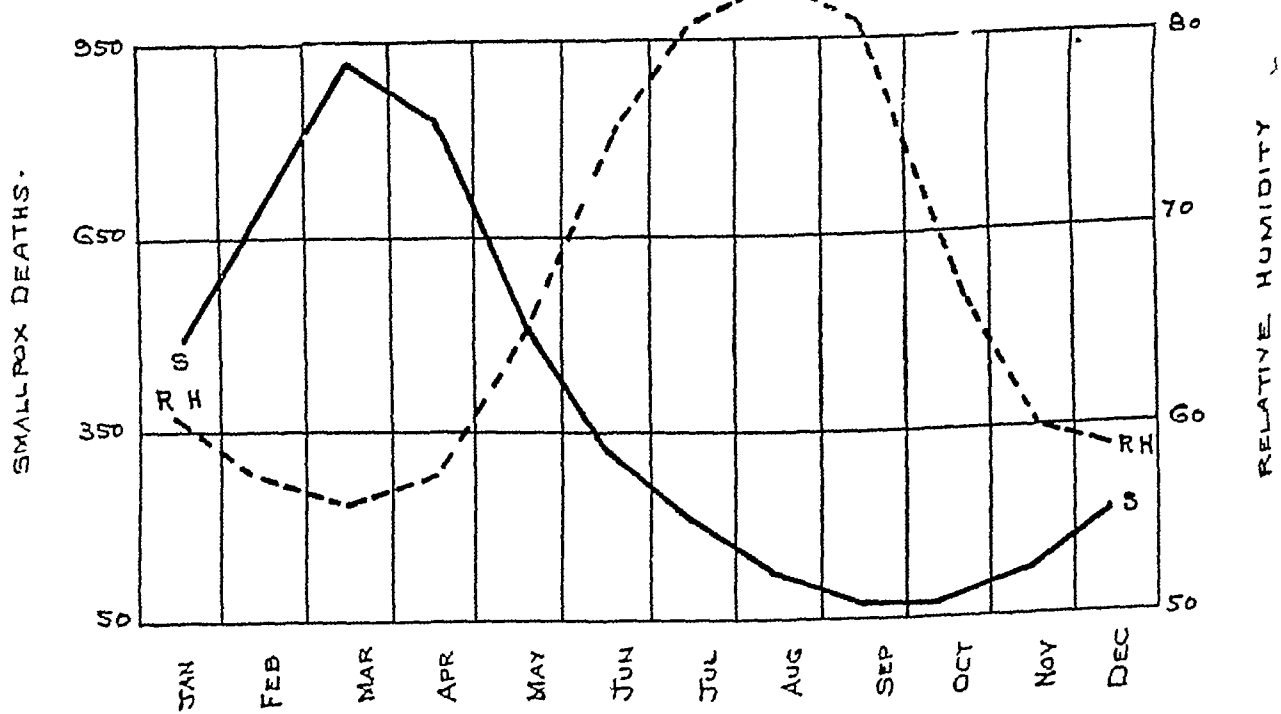


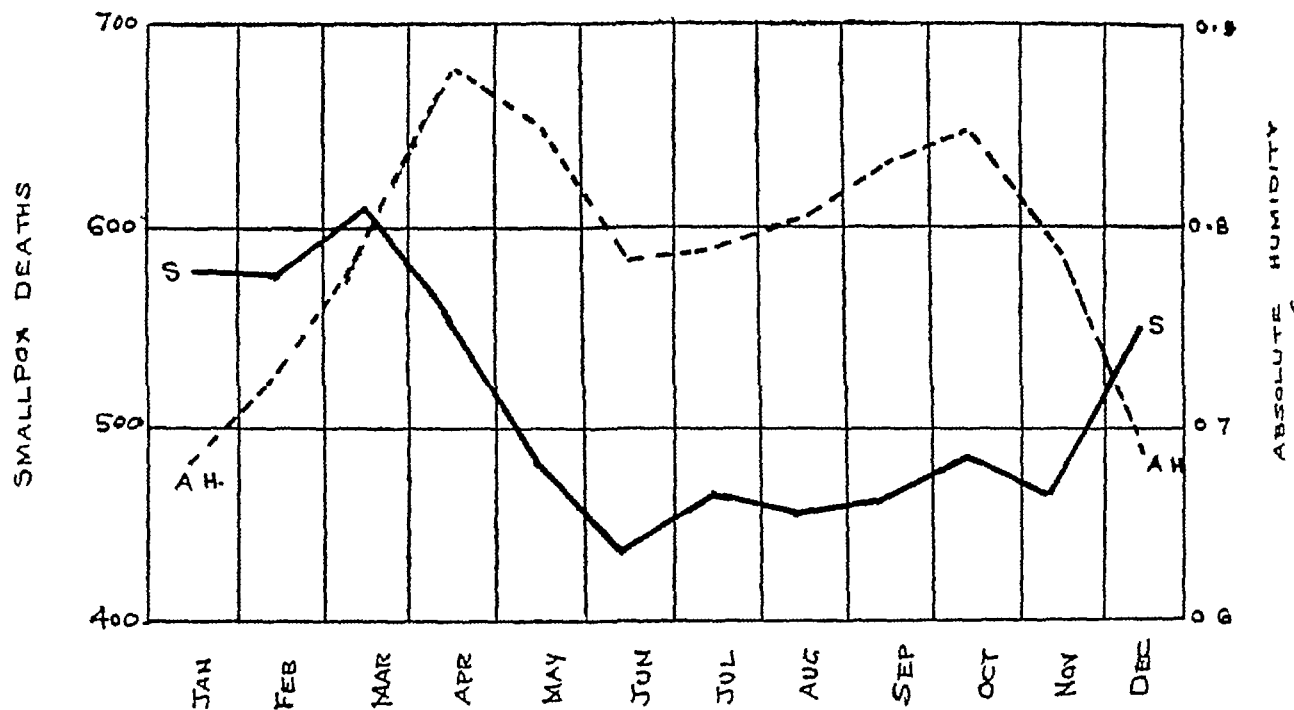
TABLE X—contd

P<sub>r</sub>

$r_{12} = -0296$	$r_{12}^*3 = +0338$ $r_{12}^*4 = -0422$ $r_{12}^*5 = -0525$	$r_{12}^*34 = +01175$ $r_{12}^*35 = +00808$ $r_{12}^*43 = +01175$ $r_{12}^*45 = -00608$ $r_{12}^*53 = +00808$ $r_{12}^*54 = -00608$	$r_{12}^*345 = +00976$
$r_{13} = -1307$	$r_{13}^*2 = -1317$ $r_{13}^*4 = -01369$ $r_{13}^*5 = -01285$	$r_{13}^*24 = -1749$ $r_{13}^*25 = -01424$ $r_{13}^*42 = -1749$ $r_{13}^*45 = -1588$ $r_{13}^*52 = -01424$ $r_{13}^*54 = -1588$	$r_{13}^*245 = -1758$
$r_{14} = +0223$	$r_{14}^*2 = +0374$ $r_{14}^*3 = -0467$ $r_{14}^*5 = -0158$	$r_{14}^*23 = -01217$ $r_{14}^*25 = -00343$ $r_{14}^*32 = -1217$ $r_{14}^*35 = -00950$ $r_{14}^*52 = -0343$ $r_{14}^*53 = -0950$	$r_{14}^*235 = -1096$
$r_{15} = -0291$	$r_{15}^*2 = -0522$ $r_{15}^*3 = +0168$ $r_{15}^*4 = -0245$	$r_{15}^*23 = +00754$ $r_{15}^*24 = -0502$ $r_{15}^*32 = +00754$ $r_{15}^*34 = -0846$ $r_{15}^*42 = -0502$ $r_{15}^*43 = -0846$	$r_{15}^*234 = -0532$

GRAPH ~~XXIX~~ (M<sub>3</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY.

GRAPH ~~XXX~~ (A)

AVERAGE MONTHLY MORTALITY, FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY

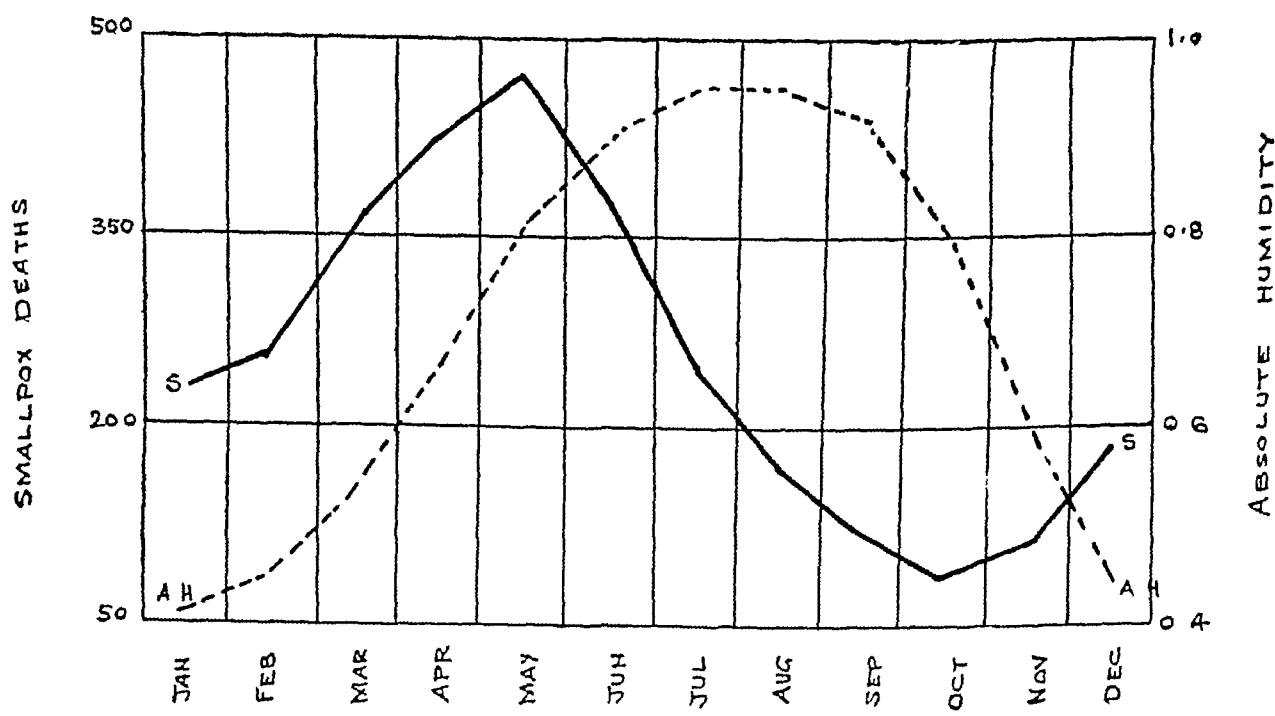


TABLE X—contd

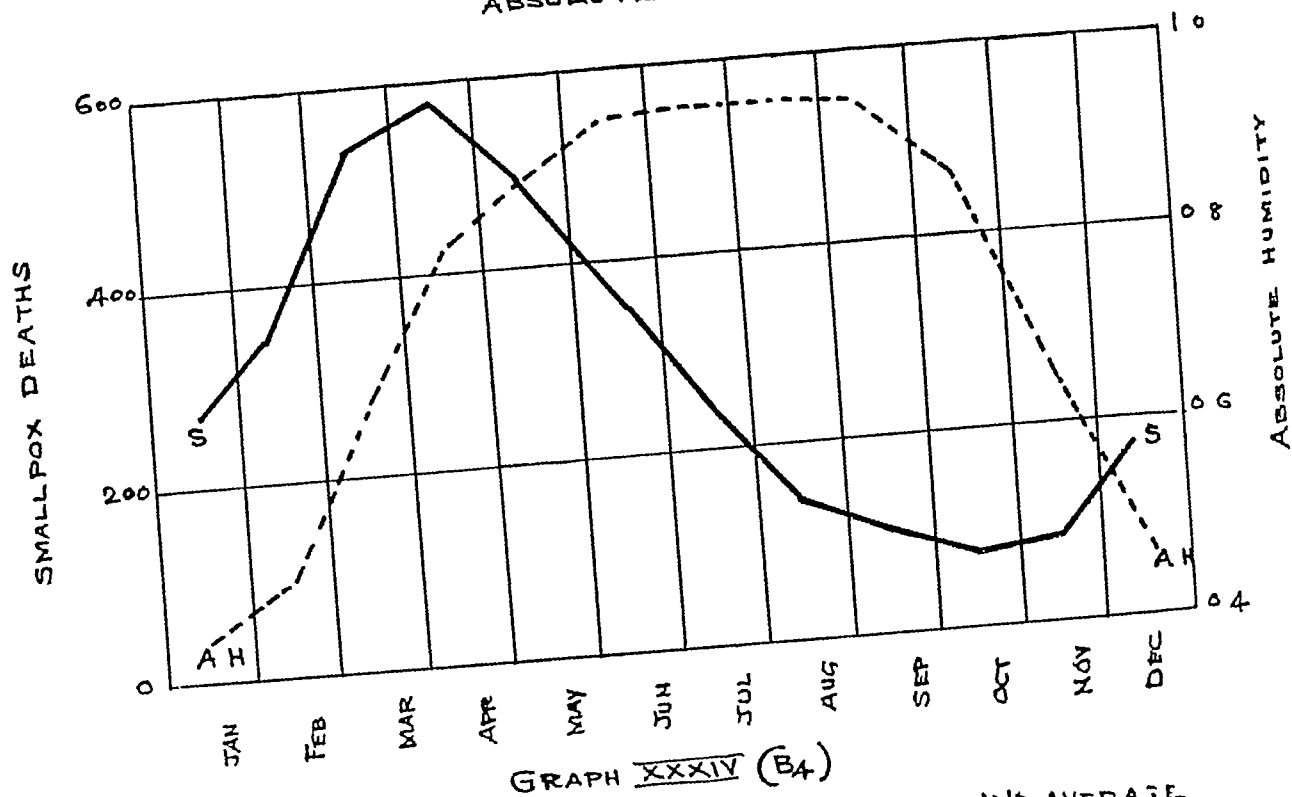
 $M_1$ 

$r_{12} = -0.1989$	$r_{12}^*3 = -0.129$ $r_{12}^*4 = -0.2124$ $r_{12}^*5 = -0.1569$	$r_{12}^*34 = +0.428$ $r_{12}^*35 = +0.807$ $r_{12}^*43 = +0.428$ $r_{12}^*45 = -0.1129$ $r_{12}^*53 = +0.807$ $r_{12}^*54 = -0.1129$	$r_{12}^*345 = +0.732$
$r_{13} = -0.2659$	$r_{13}^*2 = -0.1805$ $r_{13}^*4 = -0.2717$ $r_{13}^*5 = -0.2551$	$r_{13}^*24 = -0.1784$ $r_{13}^*25 = -0.2184$ $r_{13}^*42 = -0.1784$ $r_{13}^*45 = -0.1944$ $r_{13}^*52 = -0.2184$ $r_{13}^*54 = -0.1944$	$r_{13}^*245 = -0.1748$
$r_{14} = +0.0174$	$r_{14}^*2 = +0.0779$ $r_{14}^*3 = -0.0603$ $r_{14}^*5 = +0.1722$	$r_{14}^*23 = -0.0727$ $r_{14}^*25 = +0.1335$ $r_{14}^*32 = -0.0727$ $r_{14}^*35 = +0.0361$ $r_{14}^*52 = +0.1335$ $r_{14}^*53 = +0.0361$	$r_{14}^*235 = +0.0120$
$r_{15} = +0.1242$	$r_{15}^*2 = +0.0116$ $r_{15}^*3 = +0.0973$ $r_{15}^*4 = +0.2106$	$r_{15}^*23 = +0.1256$ $r_{15}^*24 = +0.1093$ $r_{15}^*32 = +0.1256$ $r_{15}^*34 = +0.0845$ $r_{15}^*42 = +0.1093$ $r_{15}^*43 = +0.0845$	$r_{15}^*234 = +0.1033$

# The Epidemiology of Smallpox.

GRAPH XXXIII

AVERAGE MONTHLY MORTALITY FROM SMALL POX AND AVERAGE  
ABSOLUTE HUMIDITY

GRAPH XXXIV (B<sub>4</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY

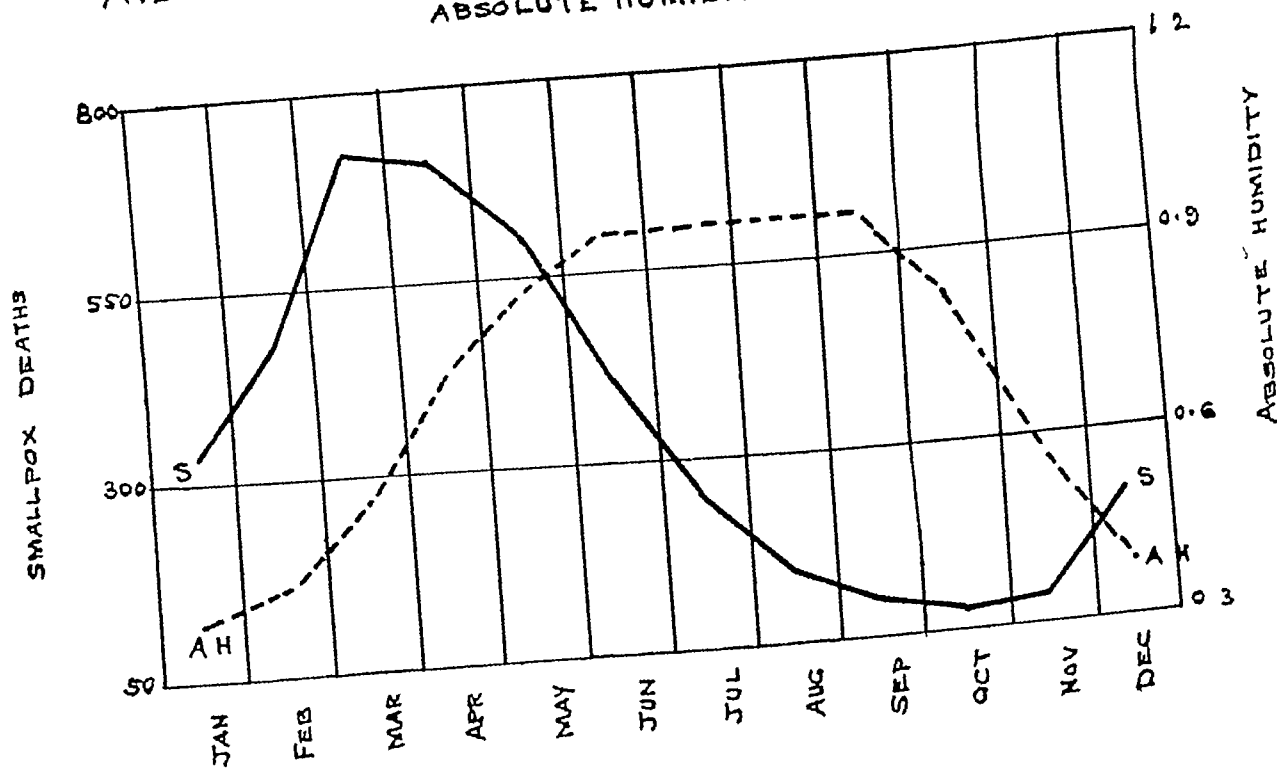


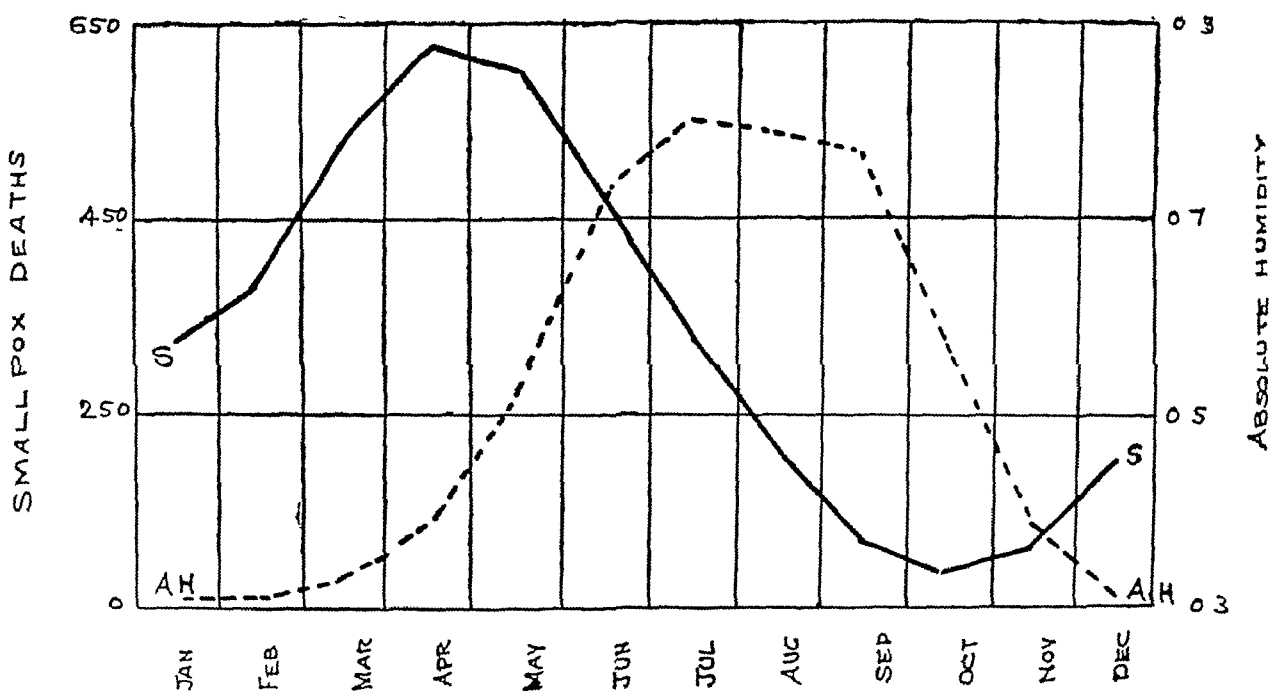
TABLE X—concl'd

 $M_3$ 

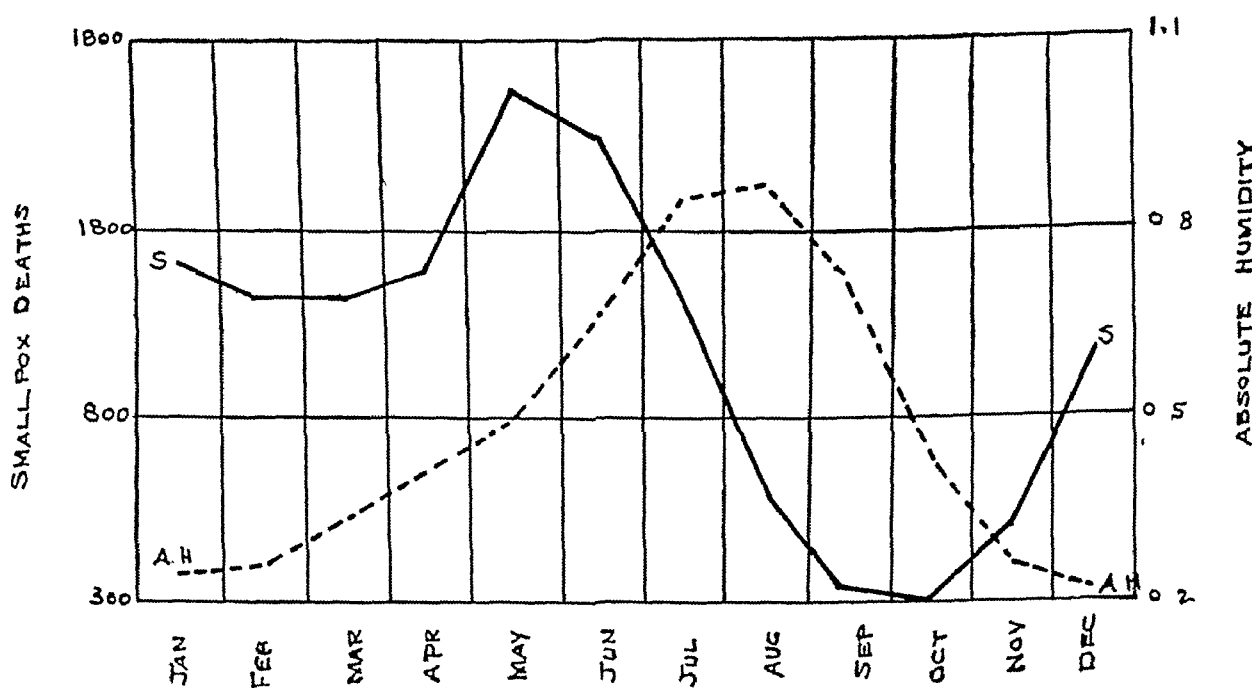
$r_{12} = -0622$	$r_{12\cdot3} = -1241$ $r_{12\cdot4} = -0844$ $r_{12\cdot5} = -0550$	$r_{12\cdot34} = -0711$ $r_{12\cdot35} = -0695$ $r_{12\cdot43} = -0711$ $r_{12\cdot45} = -0807$ $r_{12\cdot53} = -0695$ $r_{12\cdot54} = -0807$	$r_{12\cdot345} = -0594$
$r_{13} = +1406$	$r_{13\cdot2} = +1764$ $r_{13\cdot4} = -0457$ $r_{13\cdot5} = -0040$	$r_{13\cdot24} = -0022$ $r_{13\cdot25} = +0430$ $r_{13\cdot42} = -0022$ $r_{13\cdot45} = -0549$ $r_{13\cdot52} = +0430$ $r_{13\cdot54} = -0549$	$r_{13\cdot245} = -0052$
$r_{14} = -1986$	$r_{14\cdot2} = -2063$ $r_{14\cdot3} = -1487$ $r_{14\cdot5} = -0856$	$r_{14\cdot23} = -1087$ $r_{14\cdot25} = -1039$ $r_{14\cdot32} = -1087$ $r_{14\cdot35} = -1014$ $r_{14\cdot52} = -1039$ $r_{14\cdot53} = -1014$	$r_{14\cdot235} = -0948$
$r_{15} = +1816$	$r_{15\cdot2} = +1793$ $r_{15\cdot3} = +1162$ $r_{15\cdot4} = +0254$	$r_{15\cdot23} = +0539$ $r_{15\cdot24} = +0046$ $r_{15\cdot32} = +0539$ $r_{15\cdot34} = +0397$ $r_{15\cdot42} = +0046$ $r_{15\cdot43} = +0397$	$r_{15\cdot234} = +0065$

GRAPH XXXVII (C P)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
 ABSOLUTE HUMIDITY.

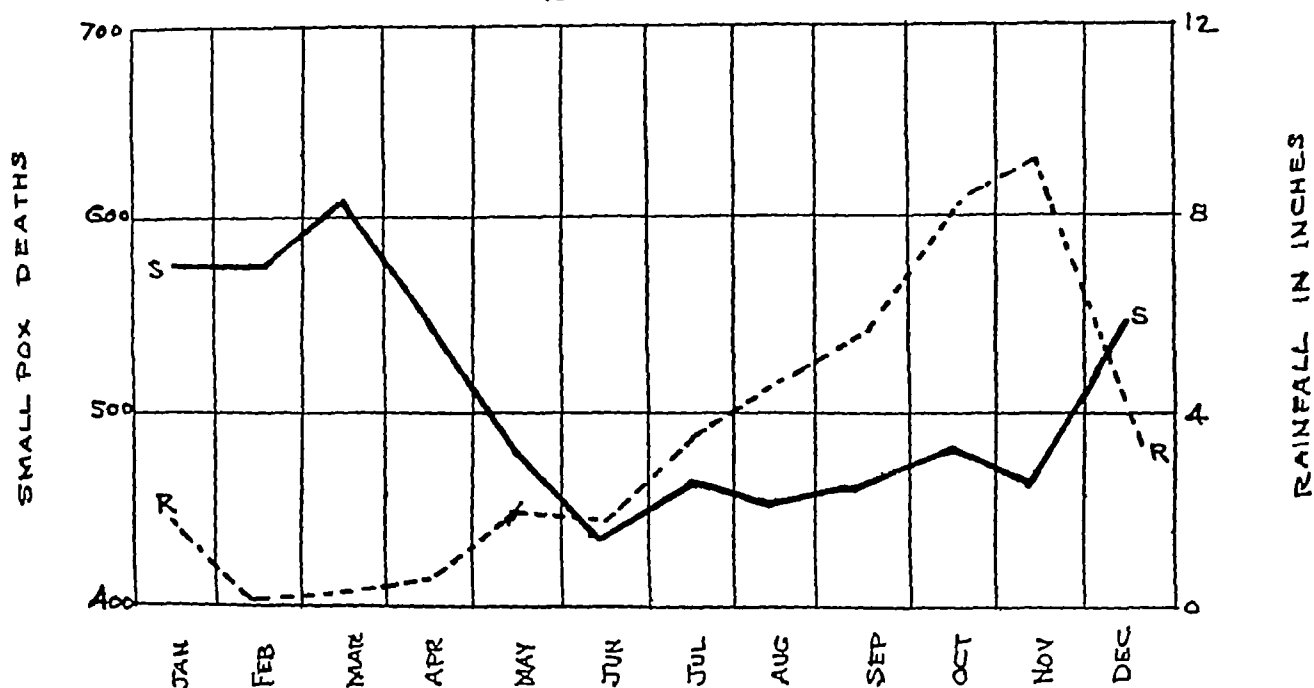
GRAPH XXXVIII (P F)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
 ABSOLUTE HUMIDITY



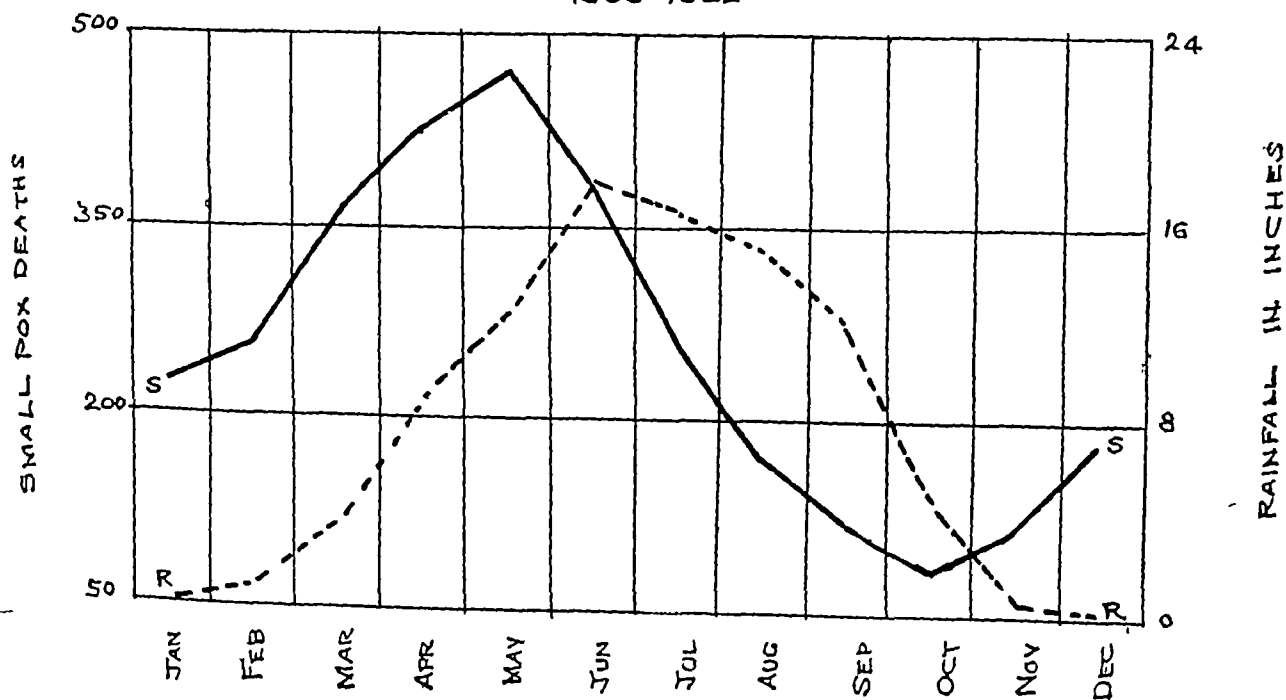
GRAPH III (M<sub>3</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD 1896-1922



GRAPH IV (A)

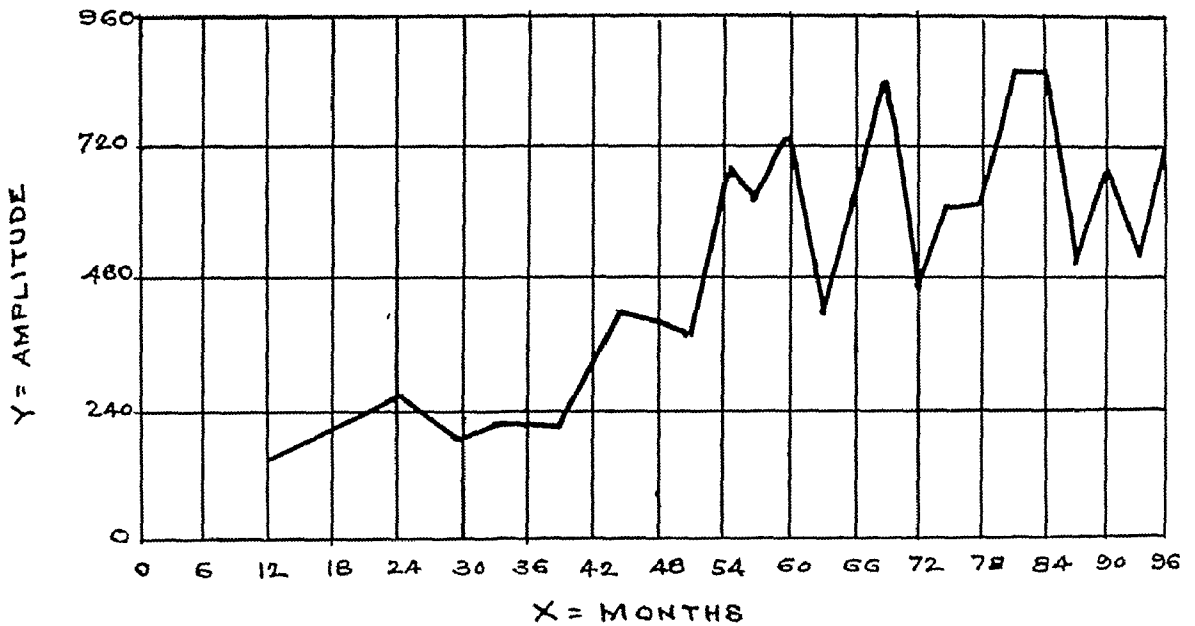
AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD 1896-1922



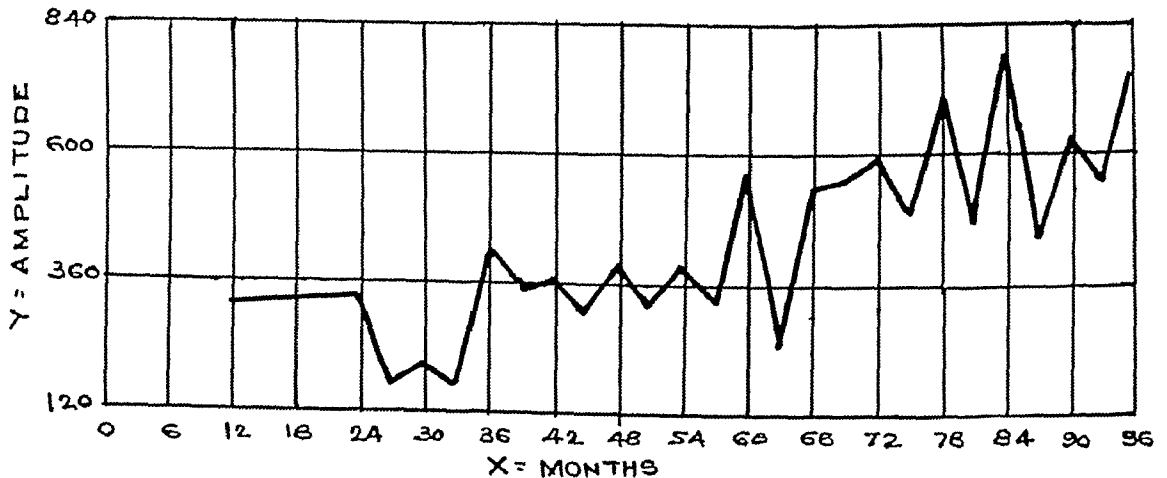


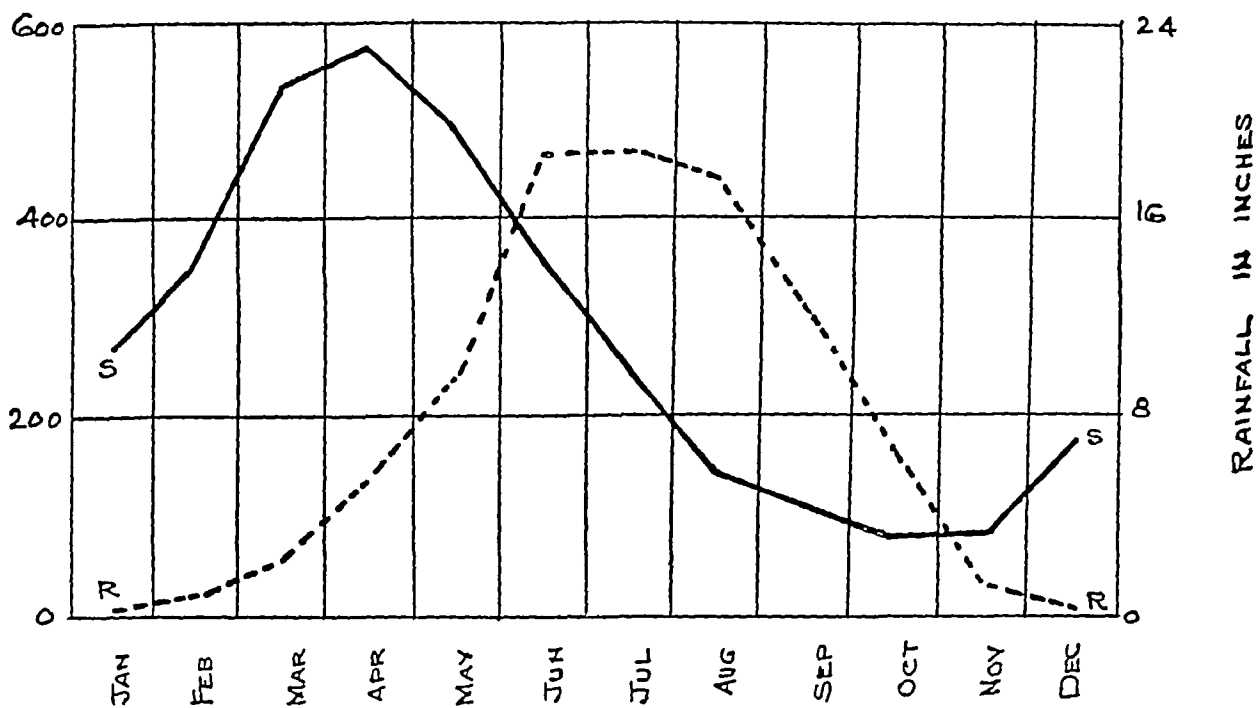
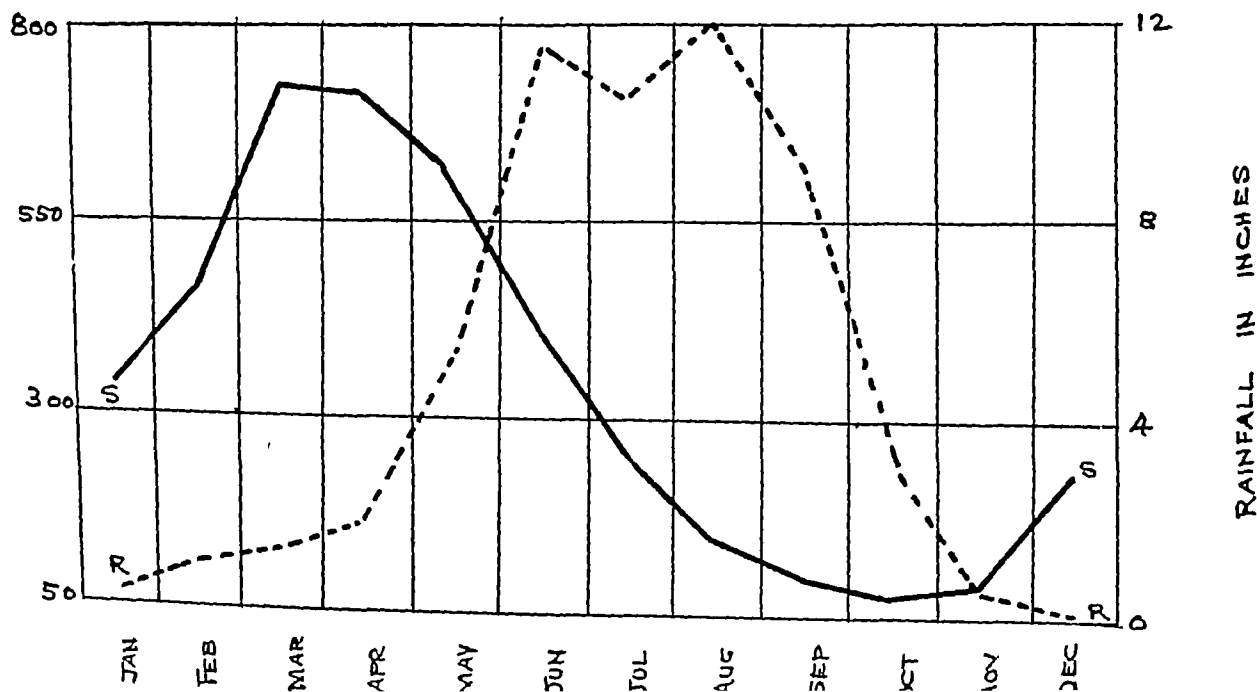
GRAPH B (M<sub>2</sub>)

MADRAS PRESIDENCY SOUTHERN DISTRICTS GROUP,  
GRAPH SHOWING MONTHLY AMPLITUDES OVER PERIOD 1866-1925

GRAPH C (M<sub>3</sub>)

MADRAS PRESIDENCY CENTRAL DISTRICTS GROUP  
GRAPH SHOWING MONTHLY AMPLITUDES OVER PERIOD 1866-1925

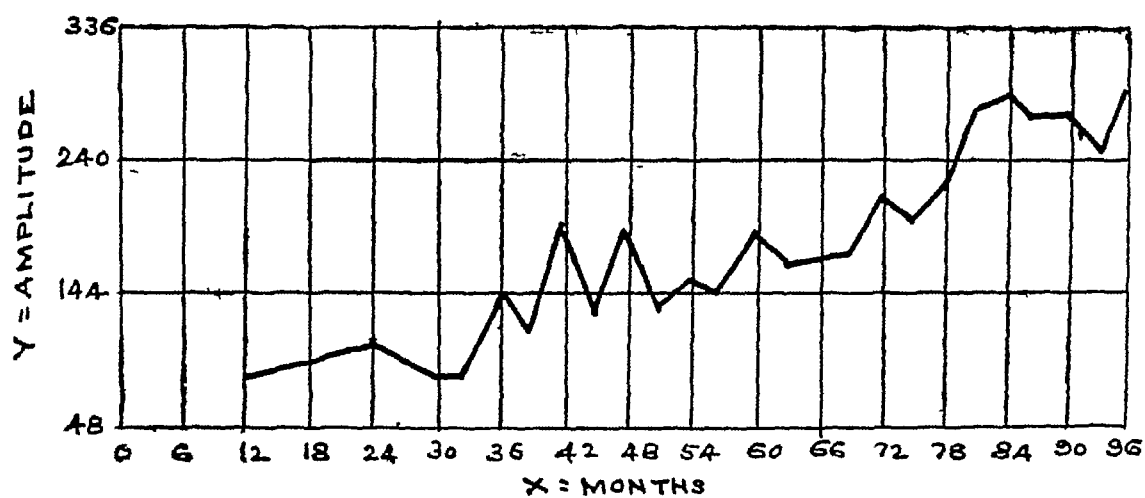


GRAPH VII (B<sub>3</sub>)AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD  
1886-1922GRAPH VIII (B<sub>4</sub>)AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD  
1896-1922

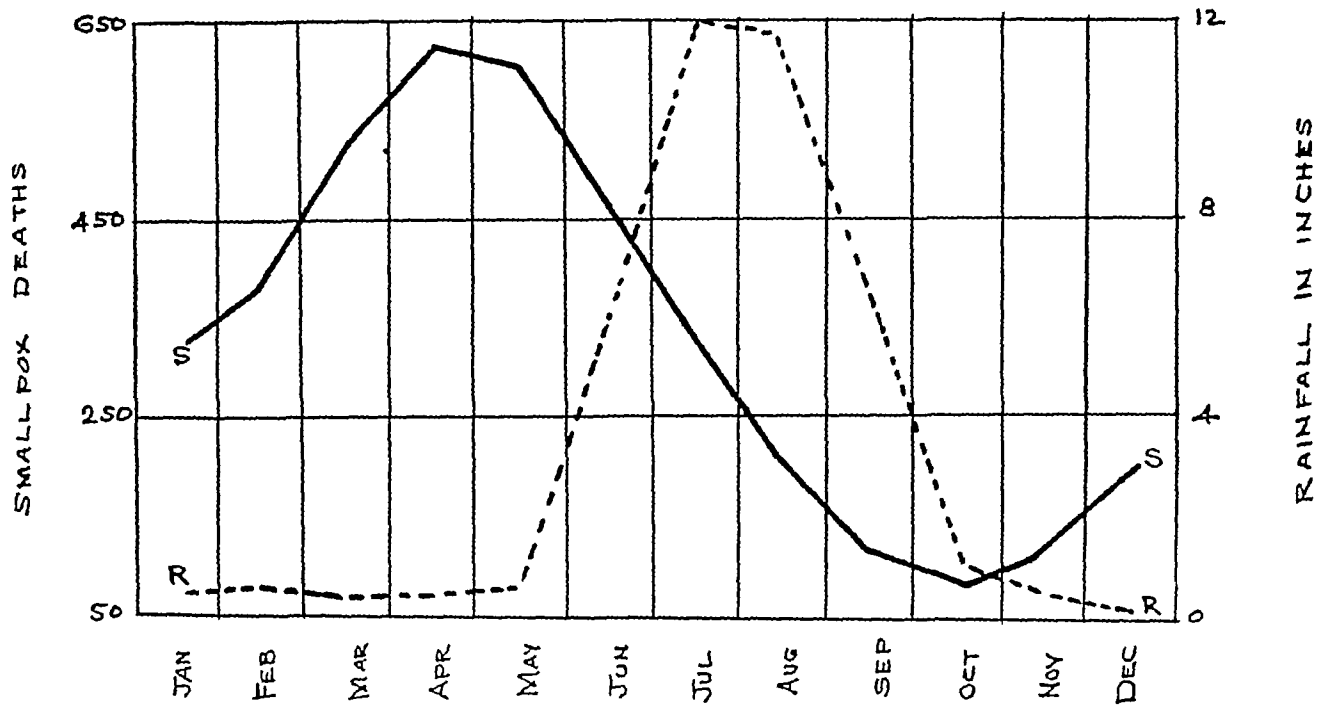
## GRAPH F

## MADRAS CITY.

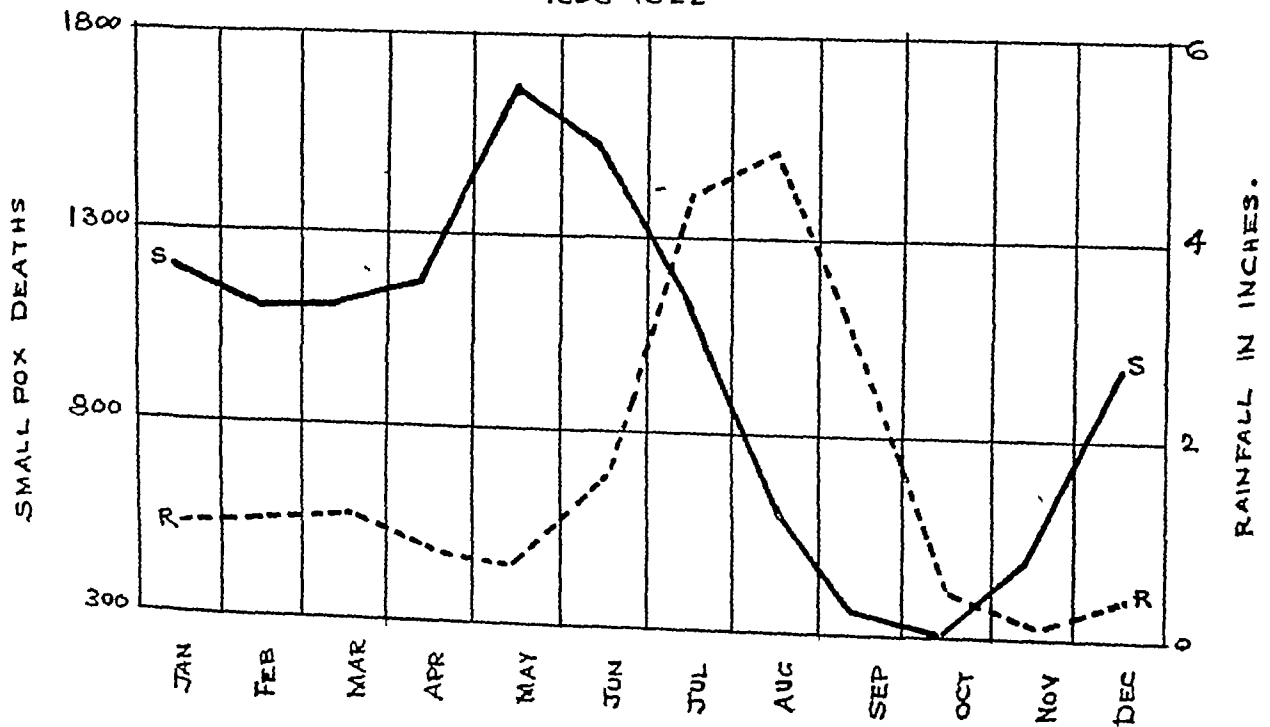
GRAPH SHOWING MONTHLY AMPLITUDES OVER THE PERIOD 1870-1925



## GRAPH XI (C P)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD  
1896-1922

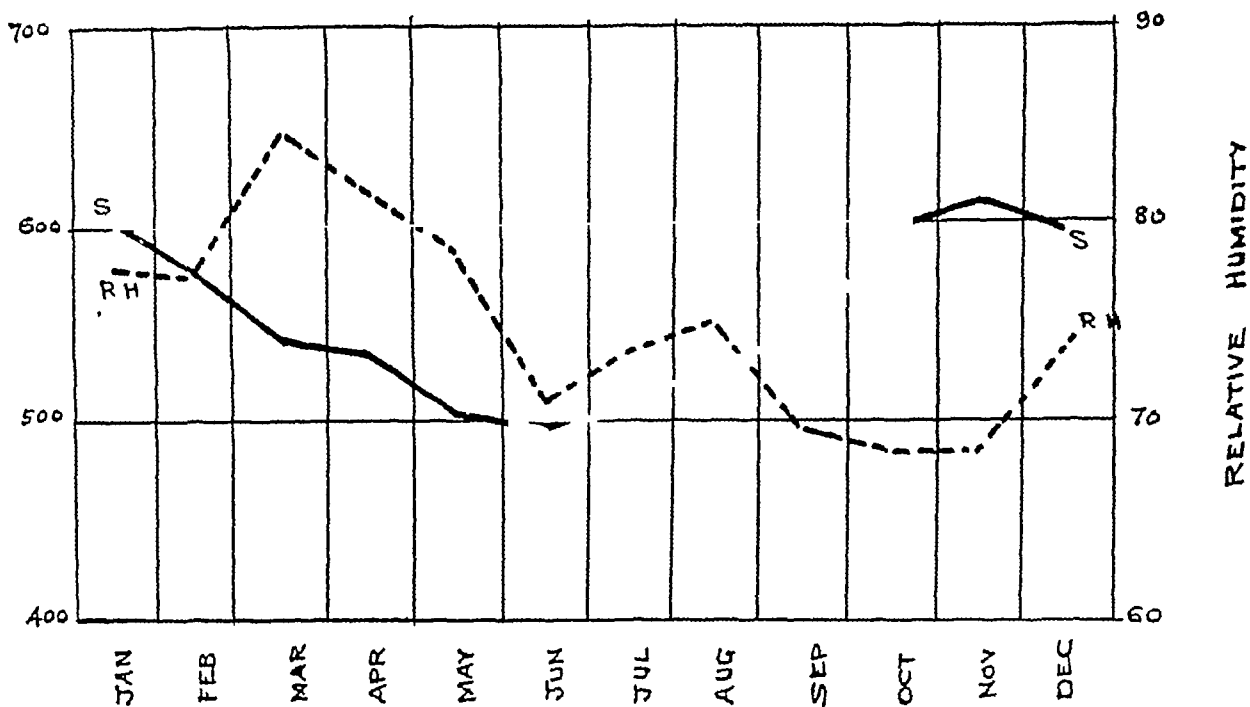
## GRAPH XII (P F)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX OVER THE PERIOD  
1896-1922



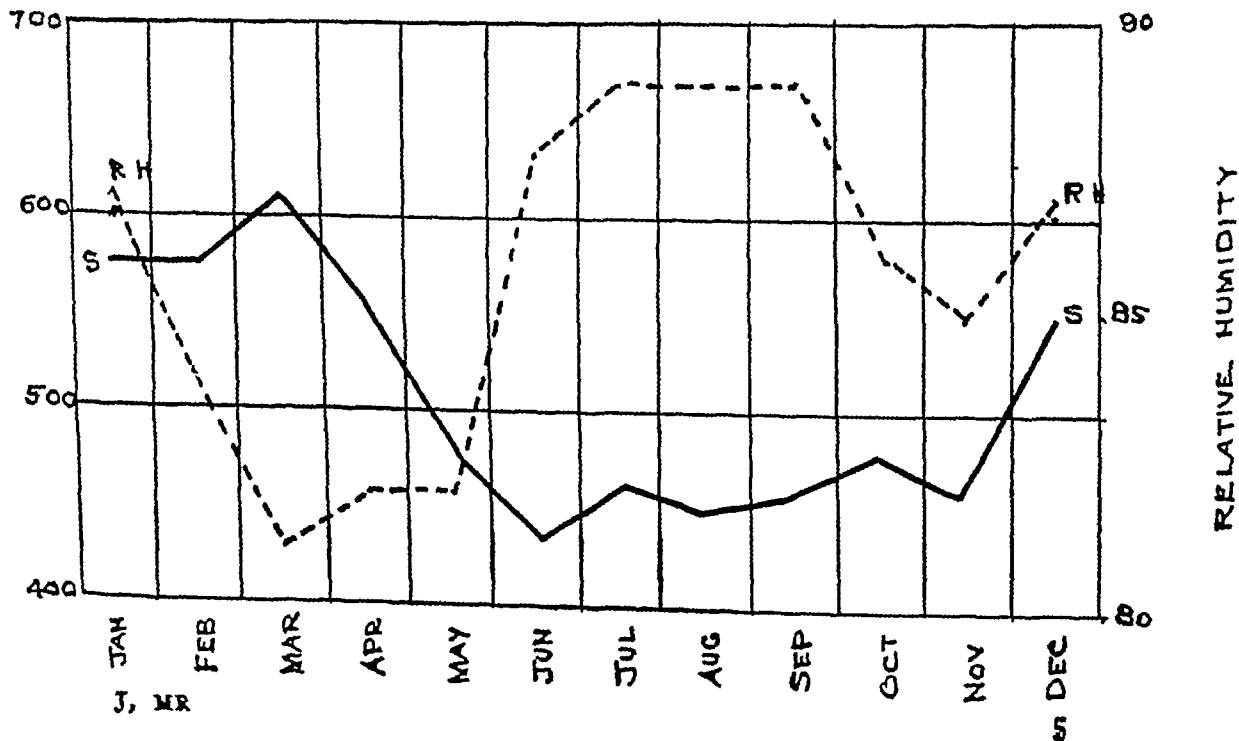
GRAPH ~~XV~~ (M<sub>2</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY.



GRAPH ~~XVI~~ (M<sub>3</sub>)

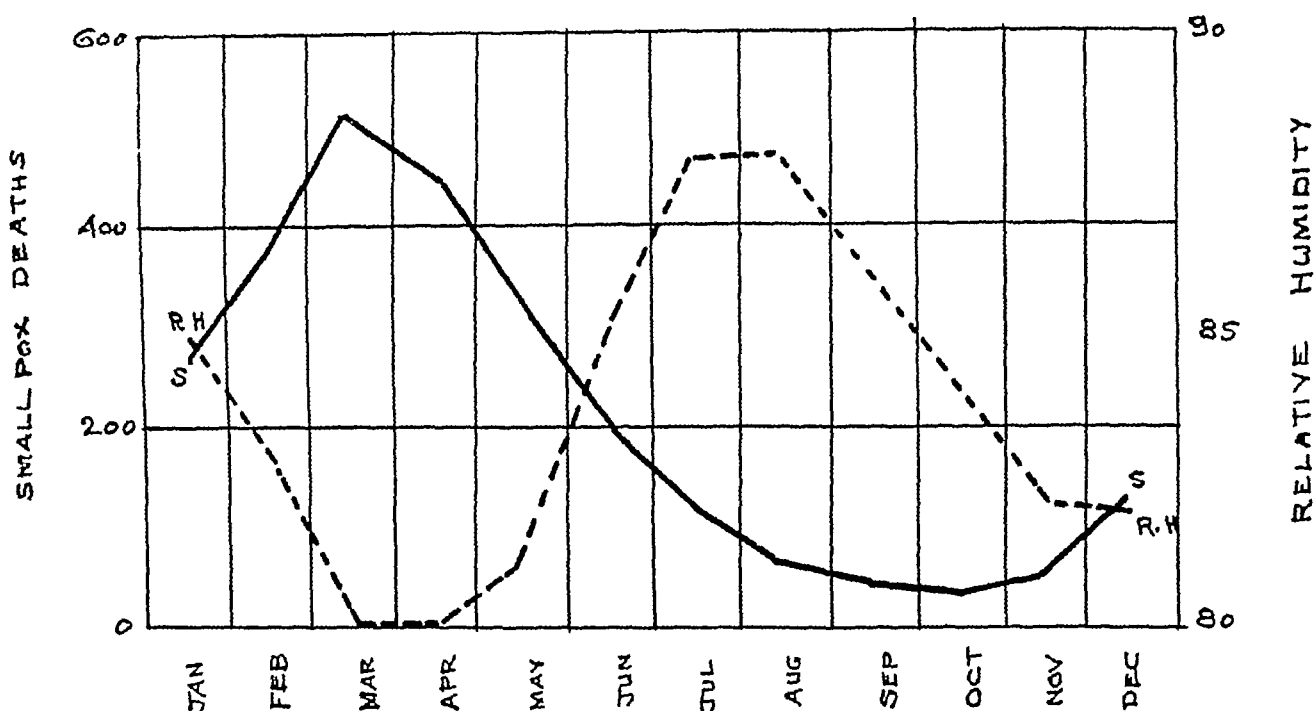
AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY



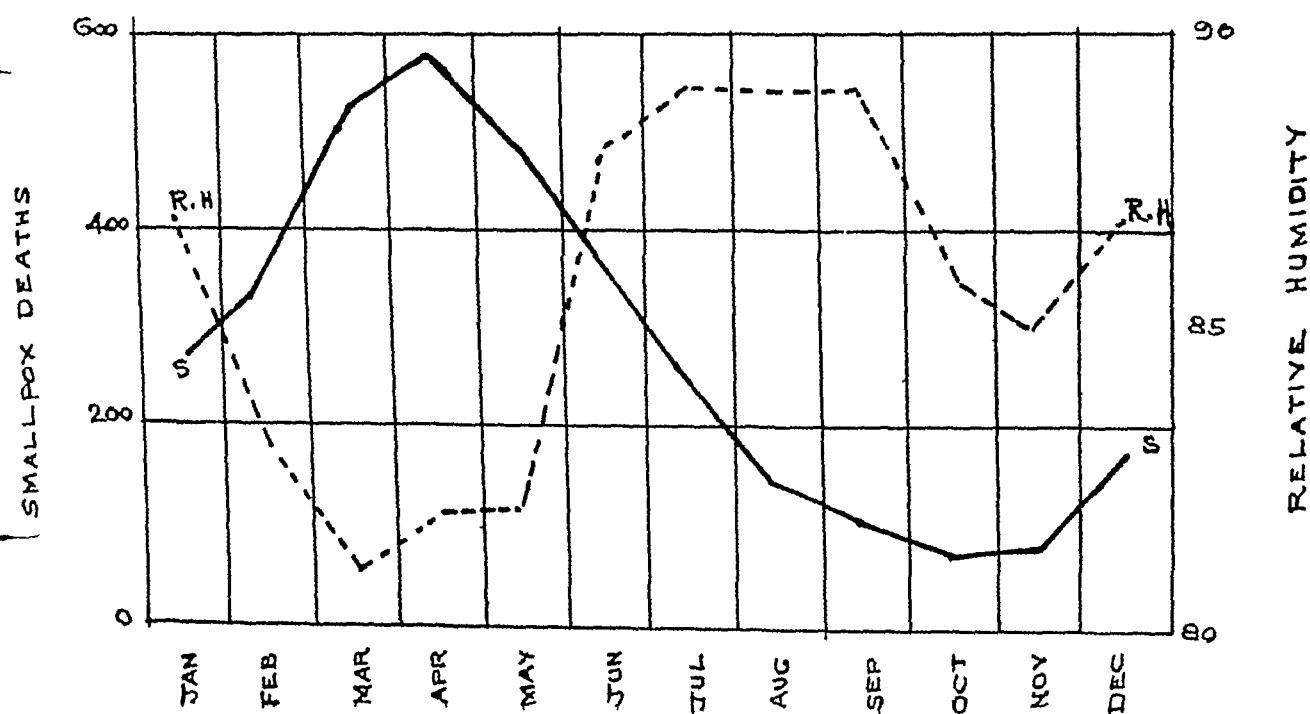


GRAPH XIX (B<sub>2</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY.

GRAPH XX (B<sub>3</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY





This work is based on the study of the larvæ from which the adults have emerged out. Individual larvæ resembling *A. fuliginosus* were isolated into separate bottles and when the larva pupated, the mouth of the bottle was plugged with cotton-wool. After the emergence of the adult mosquito, the mosquito was mounted, numbered and identified, and at the same time, the larva skin was mounted on a slide, numbered and named after the adult mosquito. Such a procedure was followed for all specimens studied. Larva skins of such of the specimens that failed to emerge out as adults were rejected. A wide range of larva skins of all three species thus definitely identified by means of emerged out adult mosquitoes has formed the material for this study. As a result of this study, it is now possible to identify even live larvæ with ease and accuracy without even placing a coverslip over the specimen. Live larvæ thus identified have been bred out into adults and the larval identification has, in all cases, been confirmed by the adult bred out subsequently. The differentiation set forth in the present article has been employed in the routine identification of larvæ collected from different parts of Bengal since the later part of 1927 and has been found to be perfectly reliable.

Detailed descriptions of the important features of the mature larvæ of *A. fuliginosus*, *pallidus* and *philippinensis* are given below and a comparative table of the characters by which they can be distinguished is given at a later stage. Photomicrographs and camera lucida drawings illustrate the different features of the larvæ of the three species.

#### LARVA OF *Anopheles fuliginosus* GILES

*General colouration*—Characteristically green in colour, and nearly transparent, the younger larvæ are dark-green or even black. The anterior dorsal region of the thorax and the 8th abdominal segment are usually marked by conspicuous snowy white patches. Head pattern is similar in all three species discussed herein, but it is particularly well seen in this species.

*Clypeal hairs*—Internal clypeal hairs with thin closely appressed branches which are few in number and not very conspicuous or pigmented. The branches of the internal clypeal hairs are less conspicuous than those of *A. pallidus* or *philippinensis*. The branches do not project far out from the main stem and are not numerous.

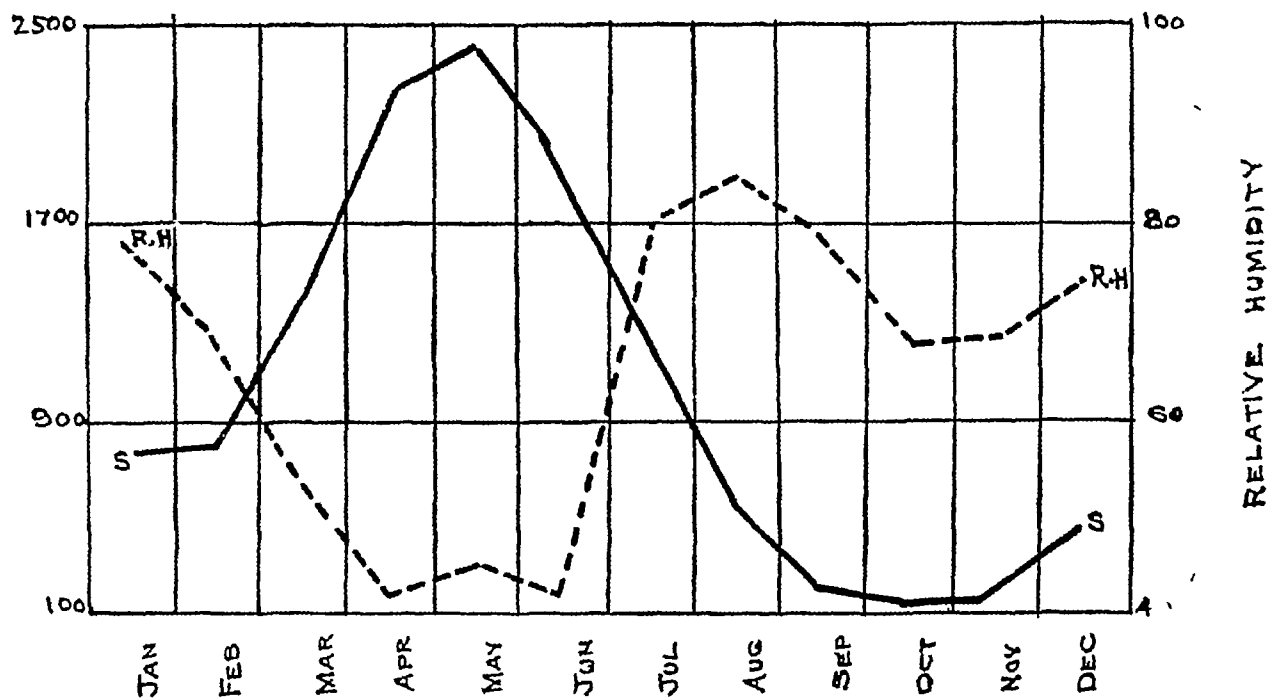
The external clypeal hairs are thin, unpigmented and usually simply branched, the branches starting pinnately from a single main rachis (Plate XLV, figs 1 and 2). The branches are thin, unpigmented and number from 13 to 16, but sometimes even less than 13. The main rachis sometimes divides into 2 or 3 branches each giving rise to subsidiary branches, but this is, however, not frequent.

The posterior clypeal hairs are pinnate, usually 3 to 4 branched and less frequently bifid (Plate XLVIII, figs  $A_2$  to  $A_5$ ).

*Occipital hairs*—The inner and outer occipital hairs are not in one line, the outer occipital hair arises distinctly anterior to the bases of the inner occipital

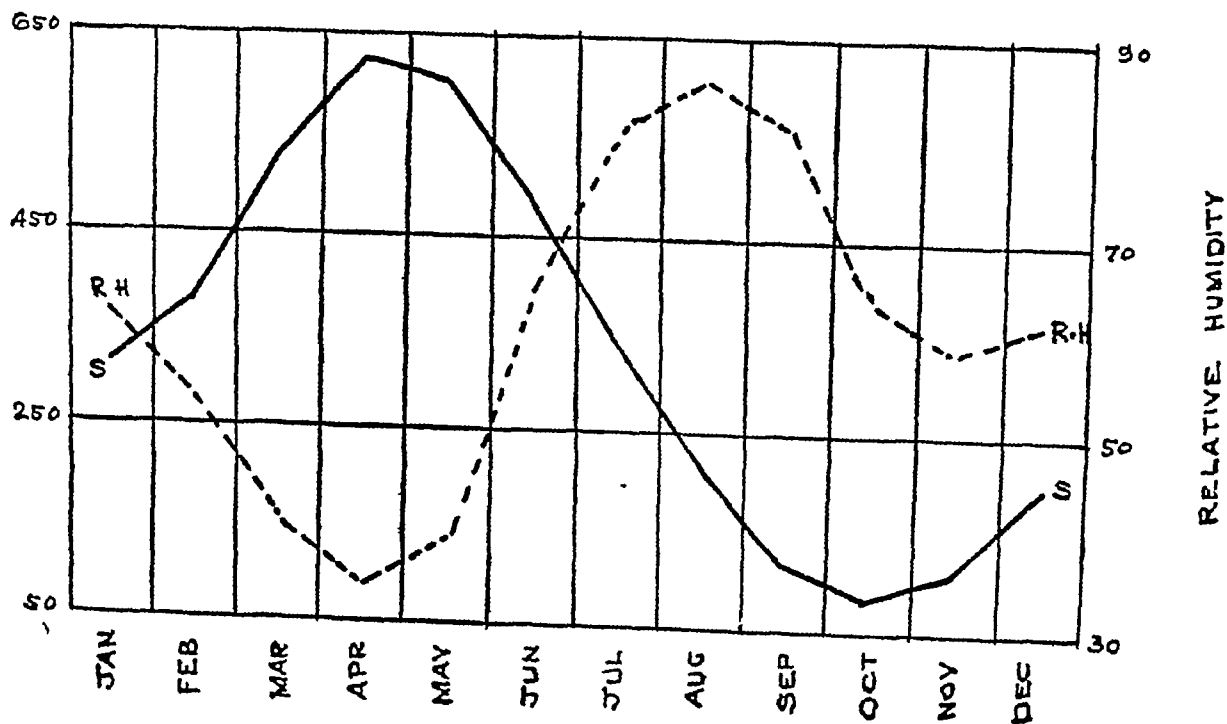
## GRAPH XXIII (U.P.).

AVERAGE MONTHLY MORTALITY FROM SMALL POX AND AVERAGE  
RELATIVE HUMIDITY.



## GRAPH XXIV (C.P.)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
RELATIVE HUMIDITY



and are spread out and not aggregated together. There are 18 to 22 branches on the external clypeal hairs, but their usual number is 20.

Posterior clypeal hairs are situated external to the line of the internal clypeal hairs and have 4 to 5 branches each, occasionally 3 (Plate XLVIII, figs B<sub>2</sub> to B<sub>5</sub>). (In very young larvæ, this hair may have only two branches.) Occipital hairs start very nearly in a row (Plate XLVI, fig 2). The inner occipital hair is dichotomously branched, the first branching being very close to the base. There are 4 to 7 branches on this hair, but usually either 5 or 6. The outer occipital hairs are pinnately branched with 4 to 7 branches, usually 5 or 6.

*Shoulder-hairs*—The internal shoulder-hair has a prominent root and a stem which is strongly chitinized and thicker than the stem of the median shoulder-hair, it is broad at middle and tapers towards base and apex. The root is well developed and prominent and is confluent with the root of the median shoulder-hair in full grown larvæ. It usually has 20 to 22 branches. The median shoulder-hair also has a well developed root, but it is not pigmented. This hair has 15 to 17 branches (Plate XLVI, fig 3).

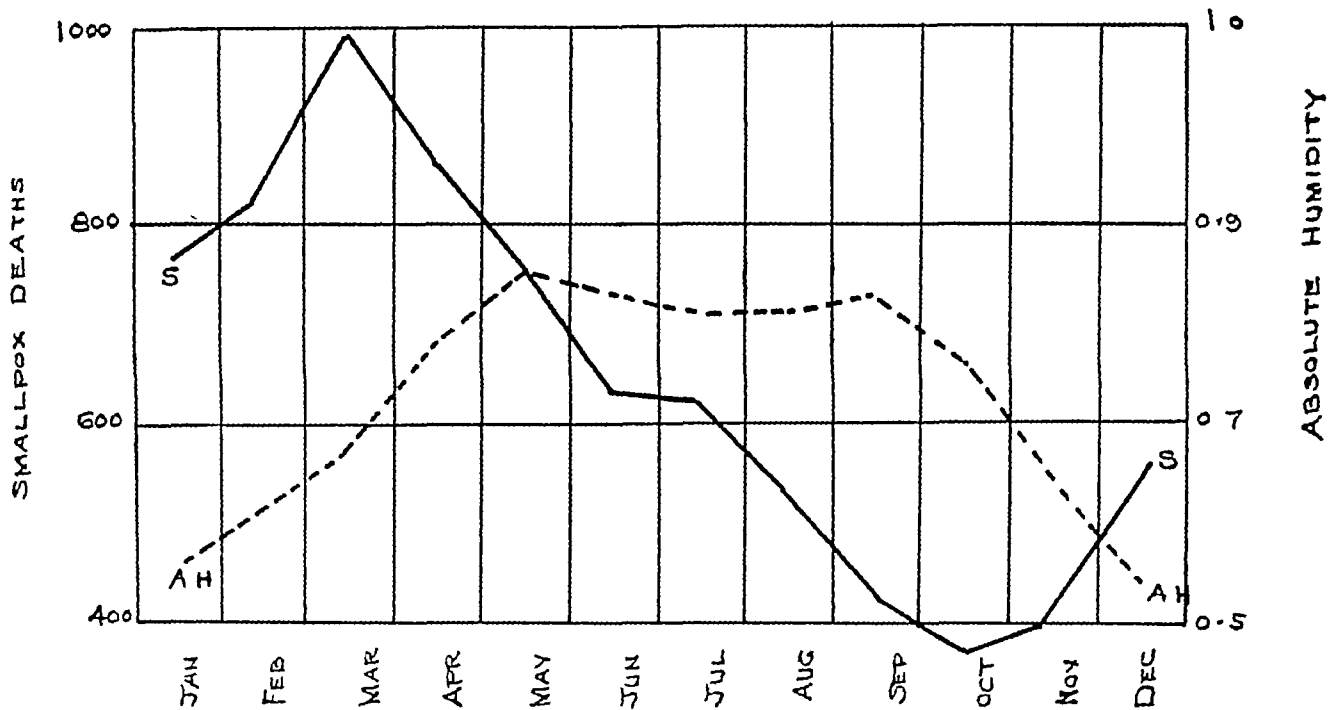
*Palmate hairs*—The thorax has a pair of well developed palmate hairs with 9 to 12 leaflets and the 1st abdominal segment has palmate hairs with 9 to 12 leaflets. In both of these, the leaflets have no filament. The 2nd abdominal palmate hair has 17 to 19 leaflets. Those on abdominal segments 3 to 6 have usually 17 to 20 blades, occasionally more. There are fewer leaflets on the 7th abdominal palmate hair, usually 15 to 18 leaflets. The leaflets of all palmate hairs show varying degrees of pigmentation, especially towards the apex of the blades and on the filaments. The pigmentation is very marked in the posterior palmate hairs, while on the palmate hairs of the thorax and the first two abdominal segments, the pigmentation though present is not so conspicuous (Plate XLVI, fig 4). The palmate hair on the 5th abdominal segment is taken as the standard for comparison. The palmate hair leaflet has a narrow blade and a filament which is thick and long, the filament is more than double the breadth of the blade. The apex and edge of the blade, as well as the entire filament, are densely pigmented in the mature larva (Plate XLVI, figs 5 and 6).

#### LARVA OF *Anopheles philippinensis* LUDLOW

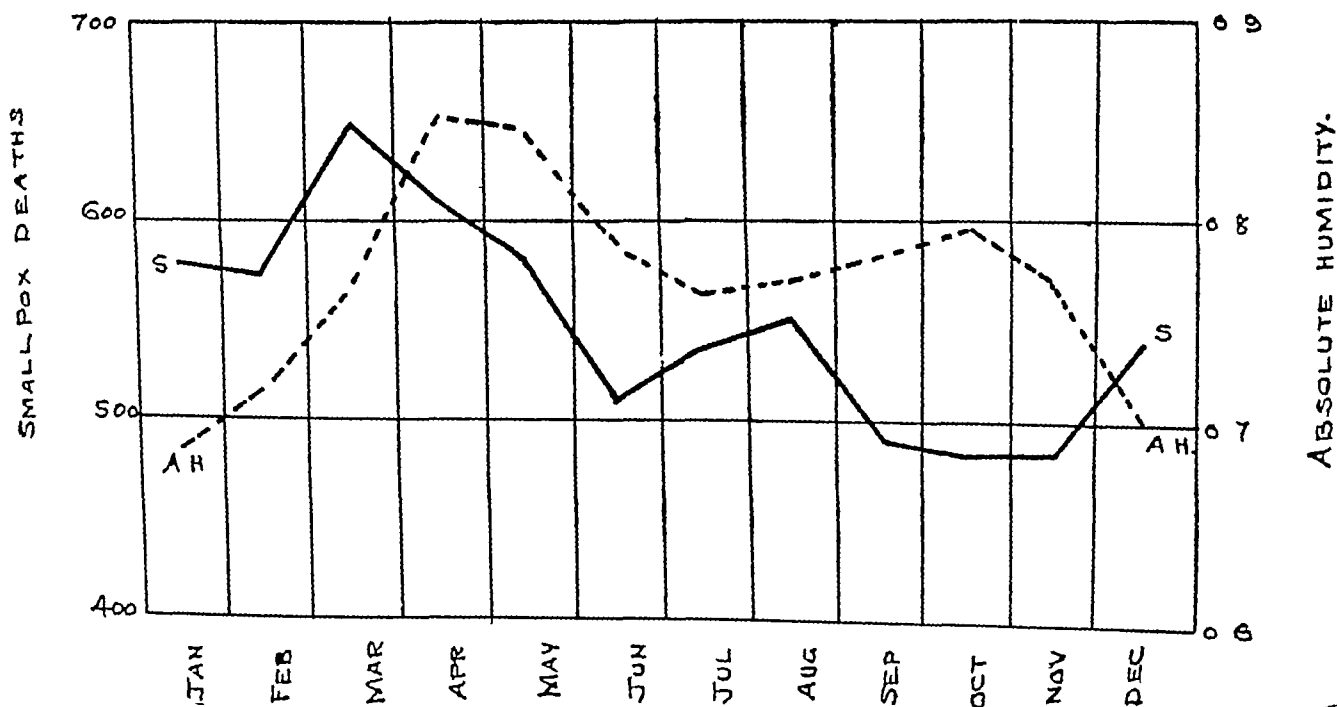
The larva of *Anopheles philippinensis* is very easily recognized by its dark-green, sometimes even black, colour and the conspicuous silver-white spots on its body (Plate XLVIII, C<sub>8</sub>). The larva has a broad white patch on the anterior border of the thorax and conspicuous white spots on the 2nd, 5th, 6th and 8th abdominal segments, respectively. Each of the spots on the 2nd, 5th and 6th abdominal segments are divided into two lateral patches by a thin longitudinal dark area. In the mature larva, this median dark area gets thinner and finally disappears, leaving the entire segment totally white. Additional but less conspicuous spots may occasionally be seen on the posterior portion of the 1st abdominal segment, and on segments 4 and 7 in the mature larva. Larvæ of this species are so characteristic in appearance that they can nearly always be picked

GRAPH XXVII ( $M_1$ )

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY

GRAPH XXVIII ( $M_2$ )

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY



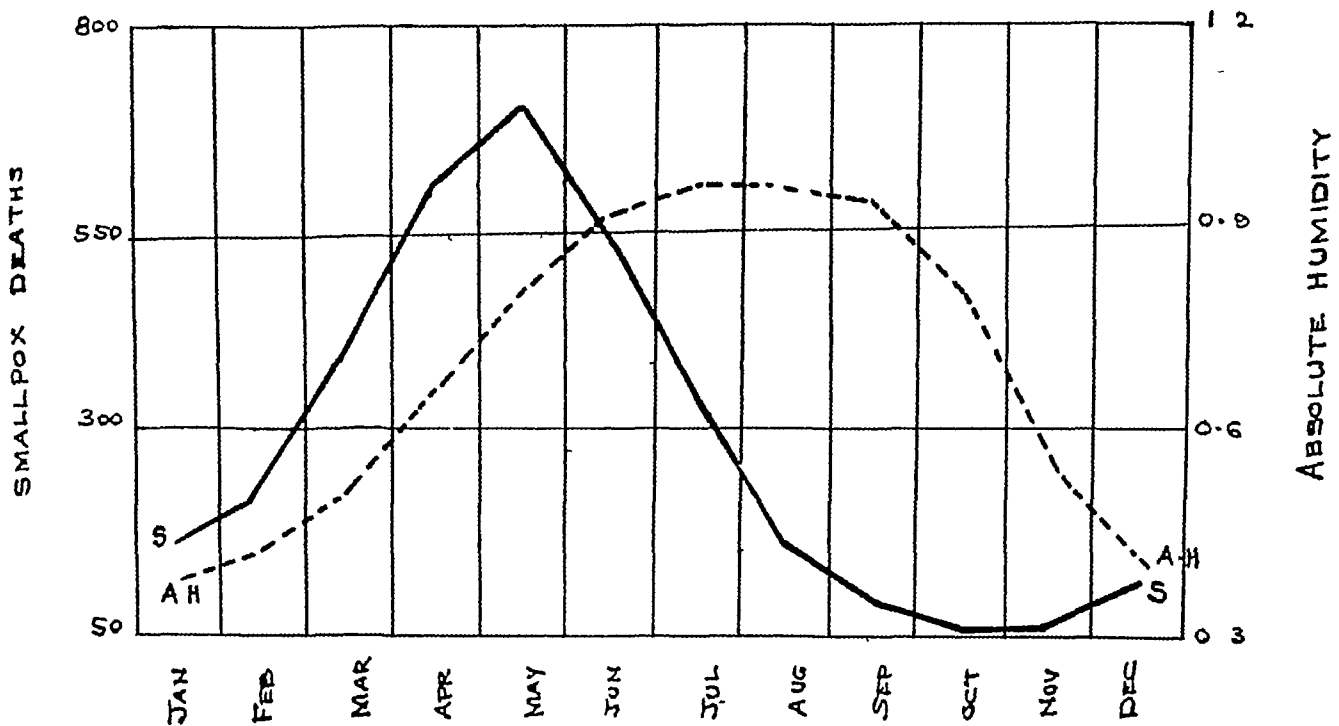
while in the two other species the filaments are equal to, or longer than, double the breadth of the blade. The blades of all palmate hairs are pigmented at apex and at sides, but not as intensively as the leaflets of *A pallidus*.

The important points of difference between the larvæ of the three species can thus be summarized as follows —

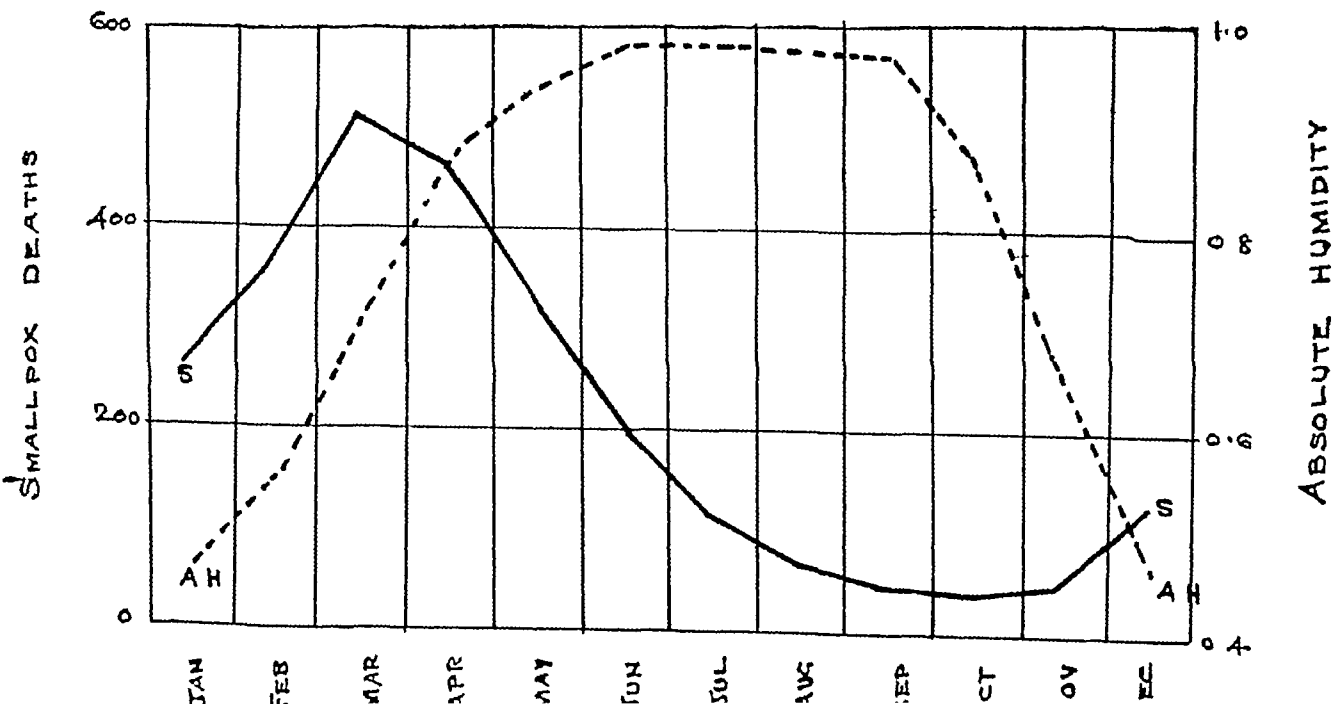
Characters	<i>A fuliginosus</i>	<i>A pallidus</i>	<i>A philippinensis</i>
White spots on body of larva	White patches on anterior portion of thorax and on the 8th abdominal segment	Usually as in <i>A fuliginosus</i> , but less pronounced	Conspicuous white patch on anterior 4th to 3rd of thorax, white spots on abdominal segments 2, 5, 6 and 8
Internal clypeal hair	Branches sparse and not conspicuous	Branches numerous and thick.	Branches thick and numerous
External clypeal hair	Generally simply branched, pinnate, branches thin, not pigmented and 13 to 16 in number	Compoundly branched, branches spread out thick, pigmented and about 20 in number	Complex branching, branches aggregated together ( <i>sinensis</i> -like), pigmented and numerous, branches usually over 25
Posterior clypeal hair	3 to 4 branched, pinnate, occasionally two branched	3, 4 or 5 branched, pinnate	Branches usually 5 to 8, sometimes more, all branches of nearly equal length, starting palmate fashion from nearly the same point close to base of hair
Internal occipital hair	Simple and long	Dichotomously branched, 4 to 7 or more first branching starts close to base	2 to 4 branches, usually 3, branches dichotomous, first branching close to base
Shoulder-hair	Pigmented, roots confluent in mature larva	Pigmented, roots confluent in mature larva	Not pigmented, roots not confluent.
Palmate hair on 5th abdominal segment.	Blade narrow or broad, filament long and thin, usually equal to or more than double the breadth of blade. Pigment absent or sparse	Blade narrow, filament long and thick, more than double the breadth of blade. Pigment on blade and filament very conspicuous	Blade broad, filament short and thin, less than double the breadth of blade. Pigment present

GRAPH XXXI (B<sub>1</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALL POX AND AVERAGE  
ABSOLUTE HUMIDITY.

GRAPH XXXII (B<sub>2</sub>)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY



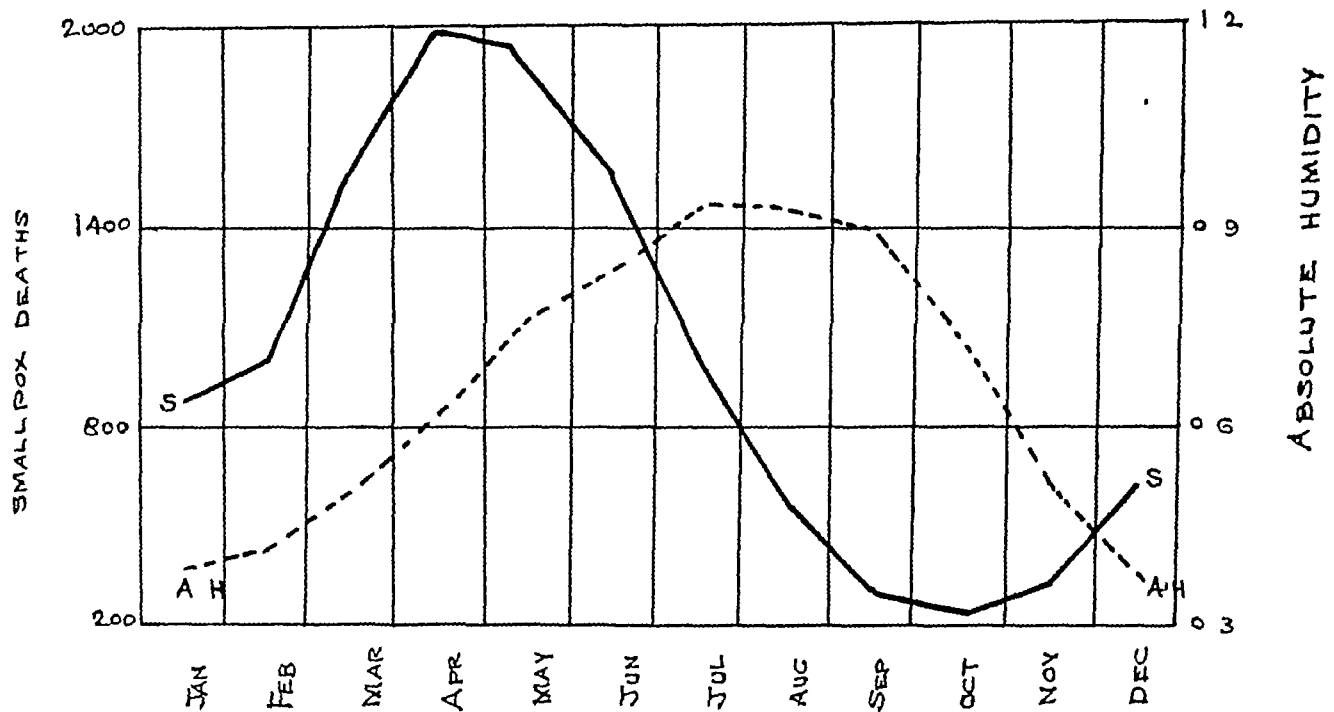
# EXPLANATION OF PLATE XLV

*Anopheles fuliginosus* Giles (Photomicrographs.)

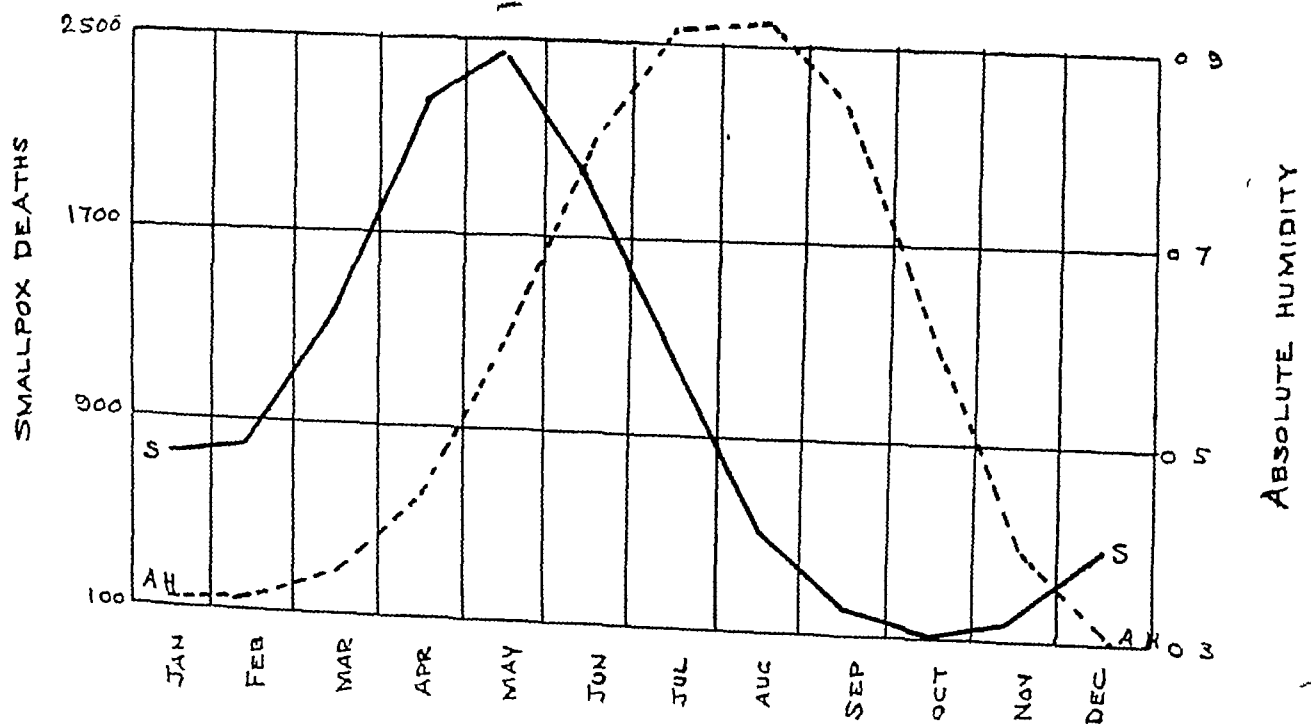
- |              |   |
|--------------|---|
| Figs 1 and 2 | Clypeal hairs of larva  |
| Fig 3        | Occipital hairs of the right side The inner occipital hair<br>(towards the left) is long and simple |
| „ 4          | Shoulder-hairs (left side)  |
| „ 5          | Palmate hairs, narrow type (segments 4 and 5)   |
| „ 6          | Palmate hairs, broad type (segments 4 and 5)  |

GRAPH XXXV (B20)

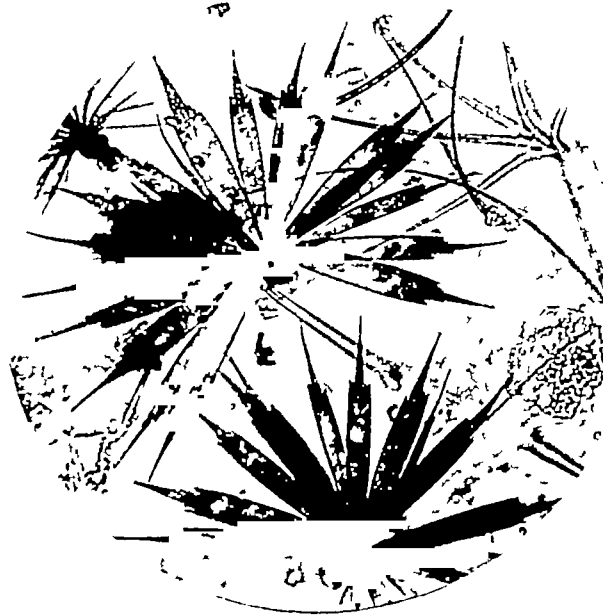
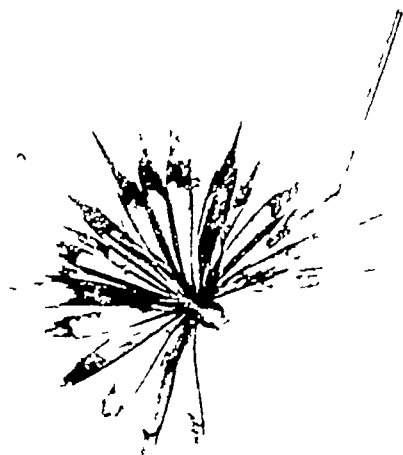
AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY

GRAPH XXXVI (U P)

AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY

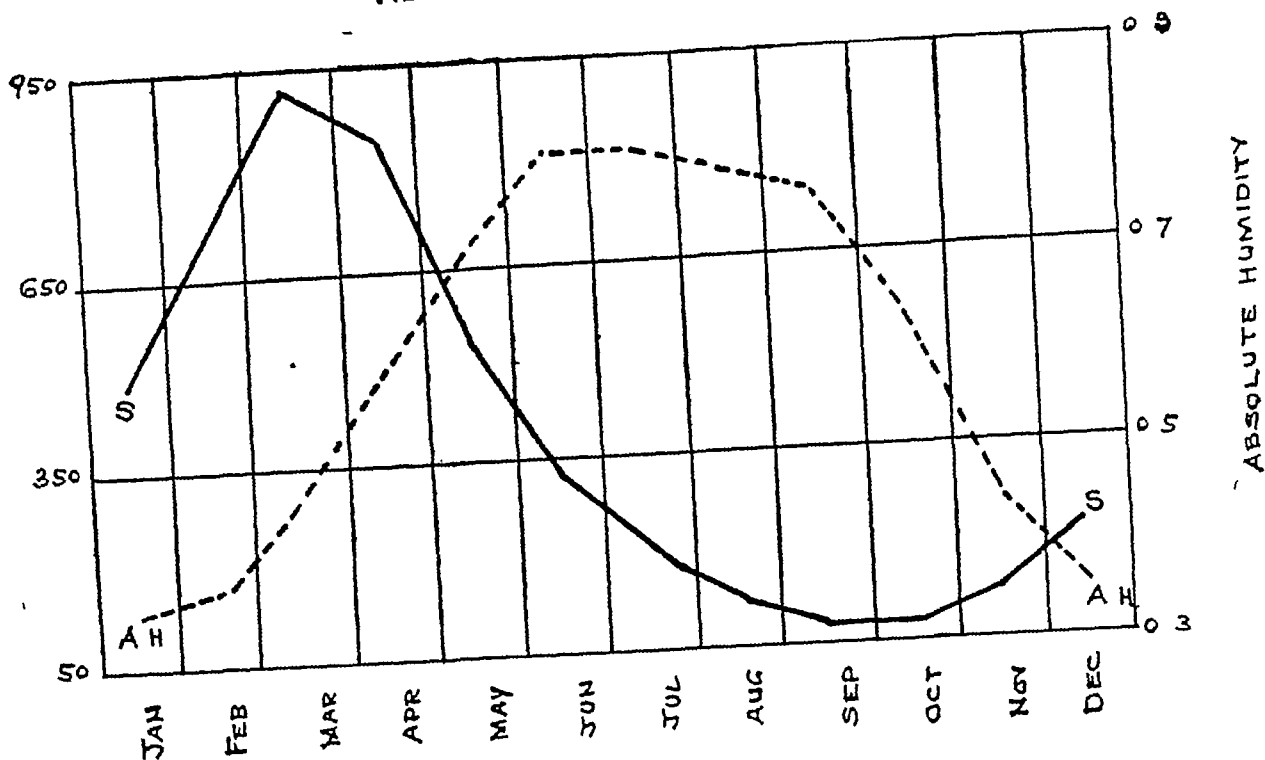






GRAPH XXXIX (BY)

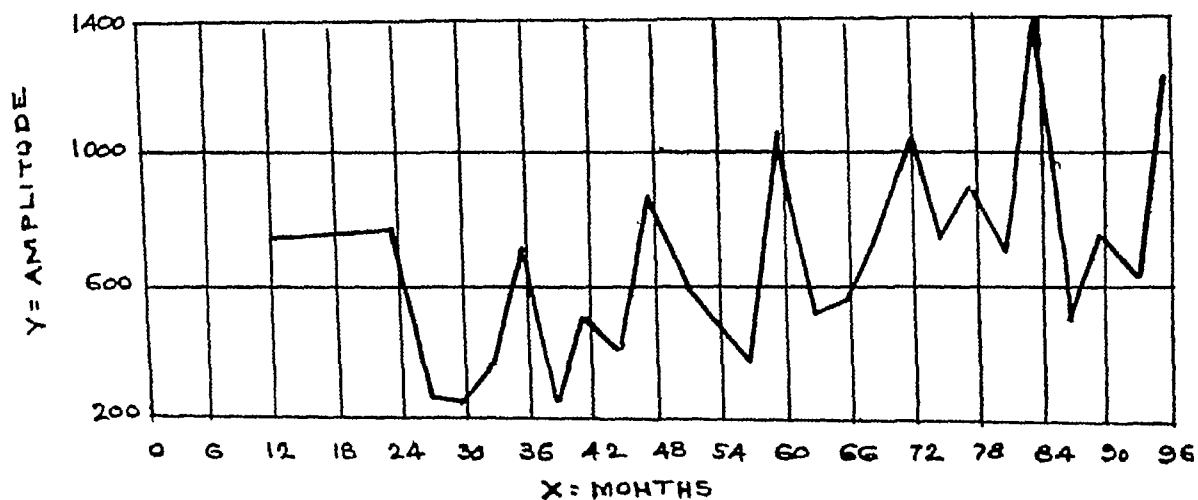
AVERAGE MONTHLY MORTALITY FROM SMALLPOX AND AVERAGE  
ABSOLUTE HUMIDITY



GRAPH A(M<sub>1</sub>).

MADRAS PRESIDENCY NORTHERN DISTRICTS GROUP

GRAPH SHOWING MONTHLY AMPLITUDES OVER PERIOD 1866-1925.



EXPLANATION OF PLATE XLVII

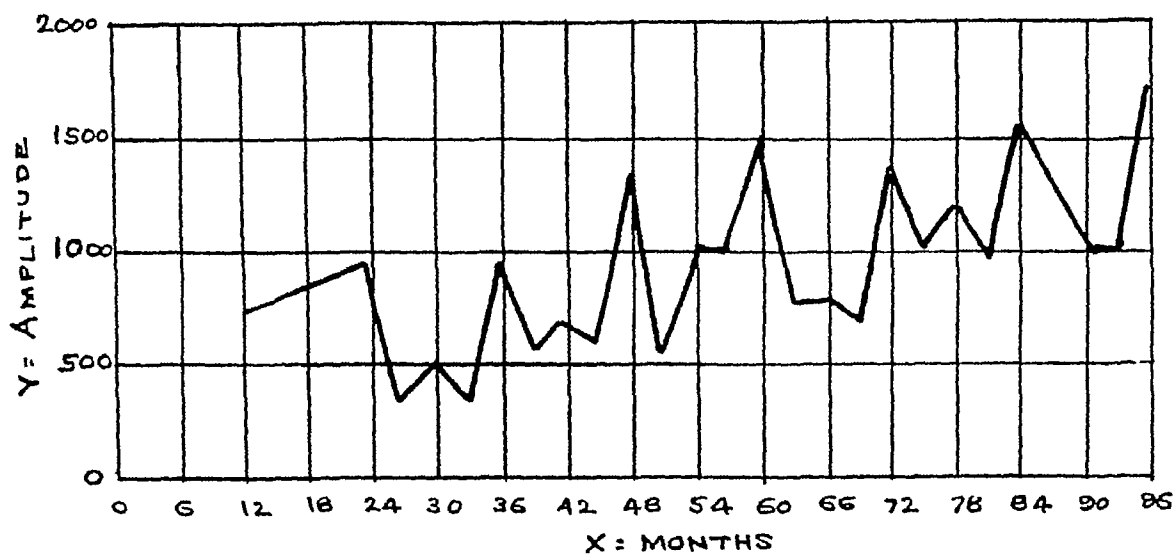
*Anopheles philippinensis* Ludlow (Photomicrographs )

- |              |                                  |
|--------------|----------------------------------|
| Figs 1 and 2 | Clypeal hairs                    |
| „ 3 and 4    | Palmate hairs (segments 4 and 5) |
| Fig 5        | Shoulder-hairs (both sides)      |

GRAPH D (By)

BOMBAY PRESIDENCY

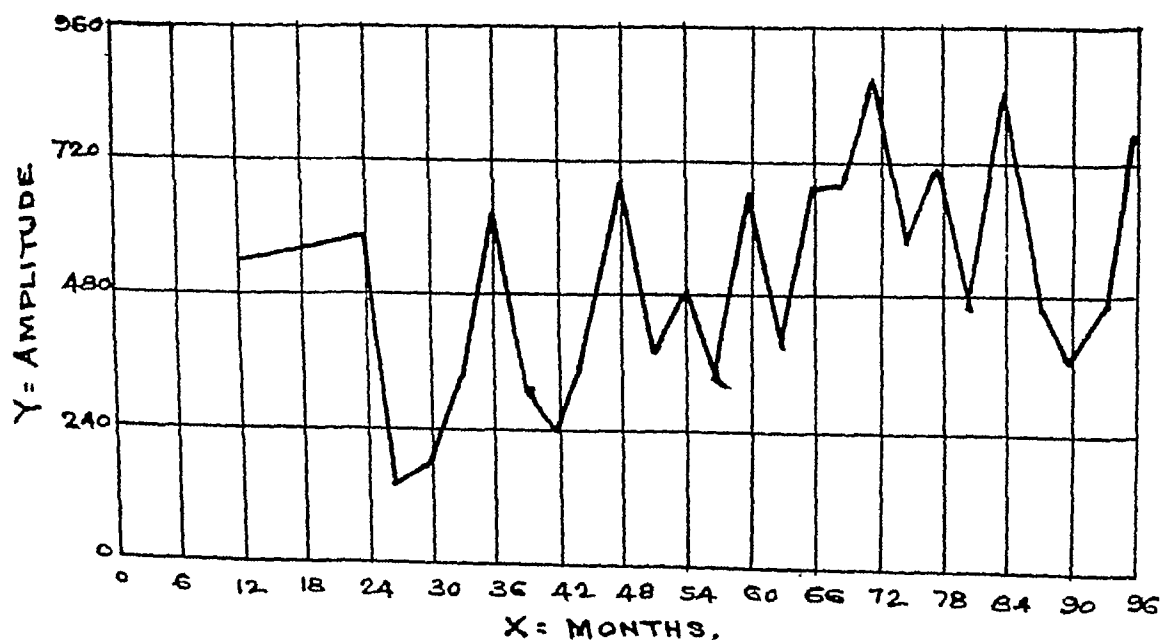
GRAPH SHOWING MONTHLY AMPLITUDES OVER THE PERIOD 1896-1922

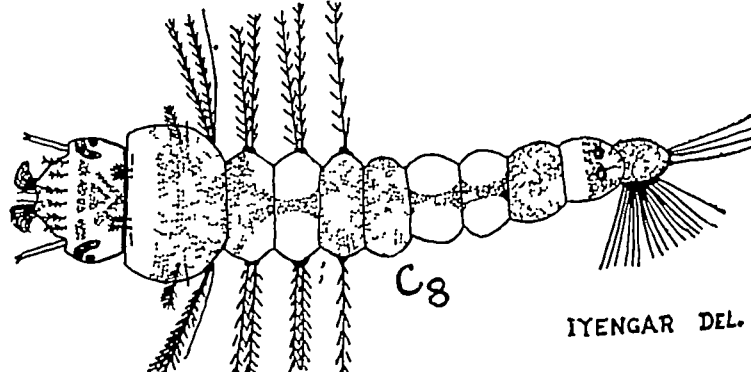
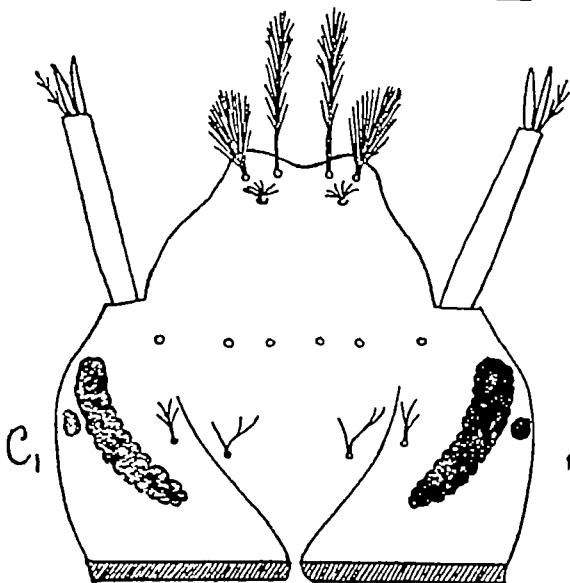
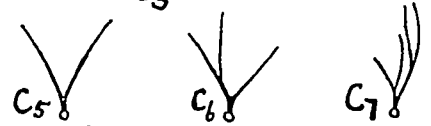
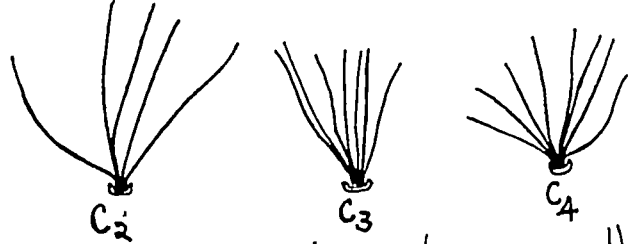
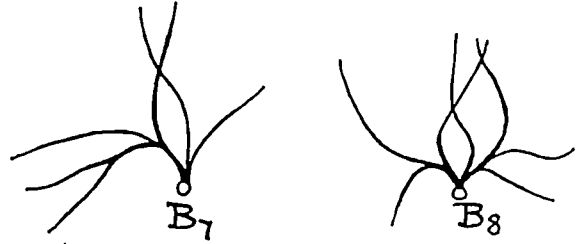
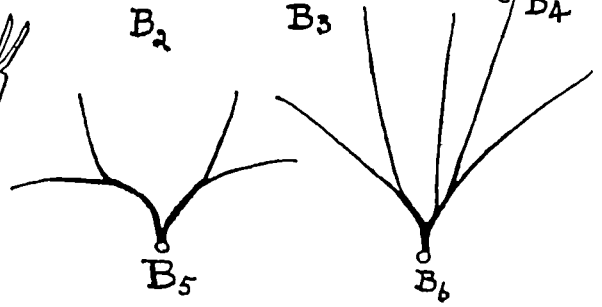
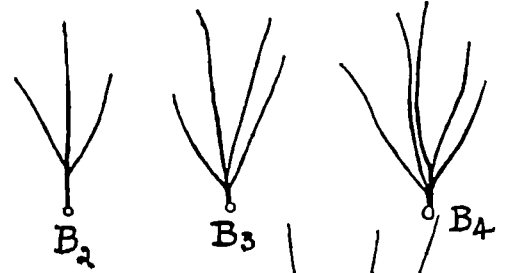
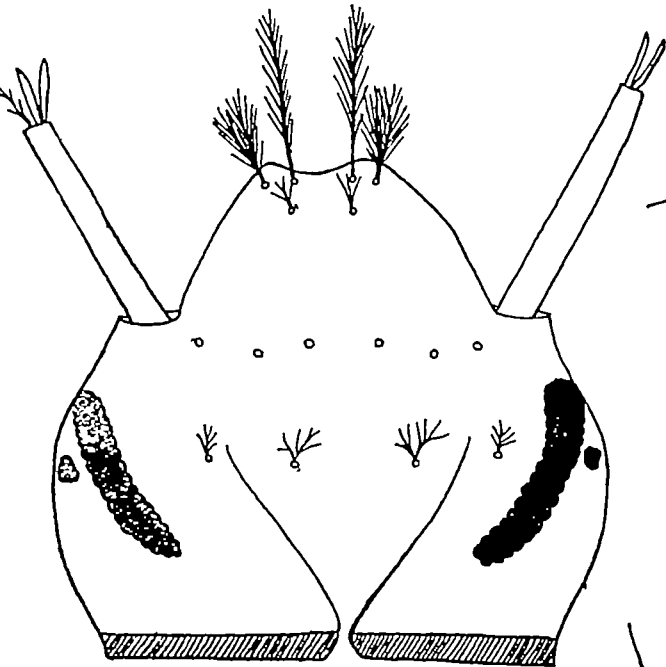
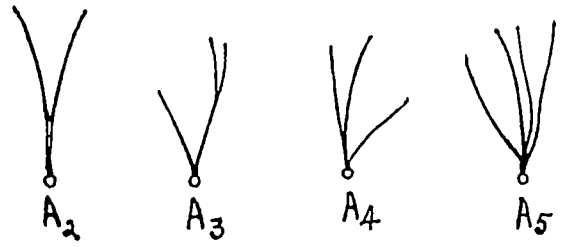
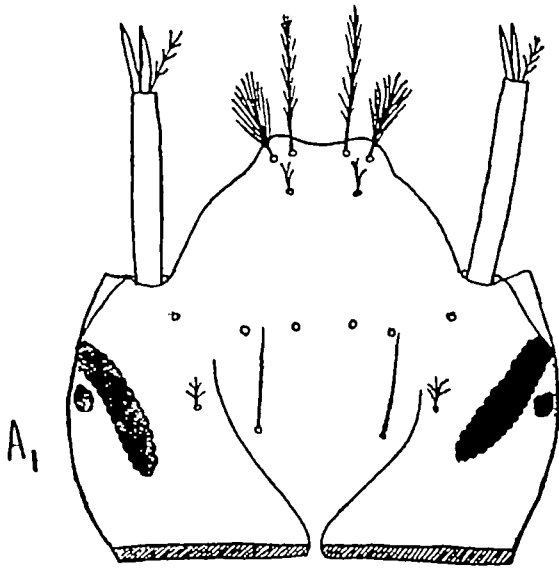


GRAPH E (C.P.)

CENTRAL PROVINCES AND BERAR

GRAPH SHOWING MONTHLY AMPLITUDES OVER THE PERIOD 1896-1922









	' r '	B <sub>4</sub>	B <sub>4</sub>	' r '	B. & O.	B. & O.	' r '
0	13	— 2006	— 1830	13·2	— 4597	— 4225	13
2	13·4	— 2216	— 1774	13 42	— 3449	— 4353	13·4
0	13·5	— 2192	— 1821	13 52	— 2977	— 4925	13·5
	13·45	— 2102	— 1781	13·452	— 3063	— 4920	13·45
1	14	+ 0225	+ 0988	14·2	+ 3357	+ 2069	14
8	14·3	+ 0986	+ 0877	14 32	+ 0937	+ 2359	14·3
2	14 5	+ 6740	+ 5535	14 52	+ 0255	+ 0915	14 5
4	14 35	+ 0389	+ 0371	14·352	— 0796	— 0879	14 35
8	15	+ 0149	— 0833	15·2	— 4338	— 5685	15
2	15 3	— 0914	— 0812	15 32	— 2489	— 3333	15 3
0	15 4	+ 0731	— 0050	15 42	— 2927	— 0354	15 4
0	15·34	— 0119	— 0170	15 342	— 2443	— 2595	15 34
8	12	— 0953	+ 0457	12·3	+ 2373	— 1302	12
7	12·4	— 1352	— 0900	12·43	+ 0972	— 2676	12·4
2	12·5	— 1256	— 1810	12 53	— 0632	— 0914	12·5

U. P.	U. P.	' r '	C. P.	C. P.	' r '	P <sub>r</sub>	P <sub>r</sub>	' r '
— 0195	— 1376	14 235	+ 0769	+ 0480	14 23	— 1217	— 1096	18
+ 0798	— 1320	14 35	+ 0728	+ 2142	14·3	— 0467	— 0950	14
+ 1792	— 0273	14 25	+ 2264	+ 2805	14 2	+ 0374	— 0343	19
+ 1401	+ 0024	14 5	+ 2817	+ 2480	14	+ 0223	— 0158	16
— 2034	— 2093	13·245	— 3450	— 3454	13·24	— 1749	— 1758	12
— 2181	— 2787	13·45	— 2813	— 3145	13·4	— 1369	— 1588	19
— 2679	— 1615	13·25	— 3990	— 4321	13·2	— 1317	— 1424	10
— 2455	— 2476	13 5	— 3844	— 3371	13	— 1307	— 1285	10
+ 0953	+ 0406	12·345	+ 2153	+ 2149	12·34	+ 1175	+ 0976	14
— 1244	— 1922	12·45	— 0570	— 1554	12·4	— 0422	— 0608	17
+ 1225	— 0067	12·35	+ 2143	+ 2966	12·3	+ 0338	+ 0808	15
— 0524	— 1902	12·5	— 1809	— 0770	12	— 0296	— 0525	10
— 1193	— 1803	15·234	+ 0607	+ 0074	15 23	+ 0754	— 0532	15
— 1702	— 1994	15 34	— 0588	— 2100	15 3	+ 0168	— 0846	17



Cultures of the cæcal contents were immediately put up in Row's hæmoglobin-saline medium (Row, 1914, Knowles, 1928, p. 653), and in this medium the parasite grew well and multiplied vigorously. The cultures became contaminated with a *blastocystis*, however, so subcultures were taken in the 'HSre-S' modification of Boeck and Drbohlav's medium, recommended by Dobell and Laidlaw (1926) for the cultivation of *Entamoeba histolytica*, with rice starch added to it to keep down the *blastocystis*. (We have found this medium to be an excellent one for the cultivation of intestinal flagellate protozoa in general, but use human serum in place of horse serum, as it appears to be impossible to secure sterile horse serum in Calcutta. The medium is particularly useful if the material is contaminated with *blastocystis*, for, on the addition of rice starch, the growth of this fungus is inhibited.) These cultures on Dobell and Laidlaw's medium, put up on the 16th May, 1928, are still growing vigorously at the time of writing—2nd July, 1928—and have provided ample material for study.

As seen in the fresh state under the dark ground microscope, this *Tricercomonas* (as we may provisionally call it) is especially characterized by (a) its very large size for a species of this genus, and (b) its sluggish movements. For both these reasons, it is a parasite which is very suitable for study with regard to the genus.

The animal is shaped like a broad ovate leaf with a more or less rounded anterior pole and a pointed, but not drawn out, posterior pole. No axostyle can be made out, either in the fresh state or in the stained preparations. Individuals vary in length from about 10  $\mu$  to 20  $\mu$ , and in breadth from 7  $\mu$  to 14  $\mu$  (exact measurements for stained specimens will be given later). Movement is in more or less of a direct line forwards, and there is no tendency to 'spin'—as with *Trichomonas*. It pushes red corpuscles and other debris in the culture fluid away from it, rather than tries to avoid them.

From a basal granule (or group of basal granules) at the anterior pole there arises a group of three flagella, which are of approximately equal length, and in length slightly shorter than the length of the body. These beat in unison and slowly. They have a kind of slow flogging action, beating upwards and then downwards and backwards on to the ventral surface of the parasite.

From the same source there arises a fourth, free and independent, trailing flagellum. This passes backwards in a postero-lateral direction (apparently across the ventral surface of the animal). As it does so, there is a tendency for the flagellum to appear as if slightly adherent to the body of the animal, but there is no trace of an undulating membrane. Finally, the terminal portion of the fourth flagellum projects behind the body in a postero-lateral direction. This flagellum beats rather more rapidly than the group of three anterior flagella, and with an independent rhythm of its own. It gives rise to a very prominent rippling appearance when seen under the dark ground.

*Stained preparations*—Films have been made, both from the fresh material from the pig's cæcum and from cultures, and have been stained by Shortt's method, in order to study the flagellar apparatus. We are much indebted to

# COMPARATIVE STUDY OF THE LARVÆ OF *ANOPHELES* *FULIGINOSUS*, *PALLIDUS* AND *PHILIPPINENSIS*.

BY

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*Entomologist, Bengal Public Health Department, Calcutta*

[Received for publication, July 6, 1928]

By reason of the close resemblance to one another of the three allied species *A fuliginosus* Giles, *A pallidus* Theobald and *A philippinensis* Ludlow, adult mosquitoes of these species have frequently been identified as *A fuliginosus*, owing to the fact that the finer details by which they are differentiated were not widely understood by medical officers engaged on malaria work in India. Although both *A pallidus* and *A philippinensis* are widely distributed in the province of Bengal, there have been barely a few published records of these two species from Bengal. Such a state of the previous records of these two species from Bengal is evidently due to the identification of these species under the name of *A fuliginosus*. The only published record of *A pallidus* from Bengal is that by Fry (1914) from the district of Murshidabad. *A philippinensis* has been recorded from only two localities, i.e., Jalpaiguri and Chittagong, [Covell (1927)] P. Sur (1928) first drew attention to the wide prevalence of *A philippinensis* in Bengal.

While the identification of even the adults of these species was not widely understood, their larvæ were too insufficiently known or probably not known at all, to enable the recognition of these species in the larval stage. These larvæ since they look so much alike, have, at least in Bengal so far as we know, been commonly identified as *A fuliginosus*. It is not always possible to breed out every larva into the adult mosquito for purposes of identification, and the identification has necessarily to be done from larvæ in the vast majority of cases. It was, therefore, essential to obtain reliable information to enable the identification of these species in the larval stage. A comparative study of the larvæ of the three species was undertaken in 1926 to enable an easy identification of these larvæ.

with one another. In his 1920 paper he describes the flagellate as having a spherical body,  $5\ \mu$  to  $6\ \mu$  in diameter, there was a nucleus, about  $1\ \mu$  in diameter, near the anterior pole. Running from the nucleus to the anterior pole was an axoneme, which terminated in a 'blepharoplast,' from which arose 3 flagella. There was no cytostome. The cytoplasm contained food vacuoles, but no other structures. Encysted forms were not encountered.

Chalmers and Pekkola (1917, 1918) recorded the finding of what they believed to be the same organism in human faeces in the Sudan, but, whereas Da Fonesca stated that two of the flagella were directed forwards and one backwards, Chalmers and Pekkola note that all three flagella are directed forwards. Chatterji (1917) encountered a similar organism in human faeces in India, but erroneously attributed it to the genus *Monocercomonas*. Later in the same year (Chatterji, 1917a) he gave a somewhat confused account of a similar organism, which he called *Trichomastix hominis*. As Dobell and O'Connor (1921) point out 'It seems clear that the organism is not, in any case, a *Monocercomonas* or *Trichomastix* (= *Eutrichomastix*)'. Later still, Chatterji (1919) described a 'new' species of '*Enteromonas*' from another case and named it *Enteromonas bengalensis*. Dobell and O'Connor remark 'It is impossible, however, from the figures and description, to identify this organism with certainty'.

M. Leger (1918) has described a similar form from man in French Guiana. Yakimoff (1925) discovered a similar flagellate in the guinea-pig and named it *Enteromonas fonescae*. Da Fonesca (1918) described a flagellate from the rabbit which he stated had a structure similar to that of his *Enteromonas hominis*, and named it *Enteromonas intestinalis*. Da Cunha and Pacheco (1923) claim to have seen both *E. hominis* and *E. intestinalis* in Brazil, and also a third *Enteromonas* in the viscacha. Lynch (1922) obtained from the guinea-pig a culture of a flagellate having a similar structure. Brug (1923) gave a description and figures of *E. hominis* from Sumatra. During the last few years in Calcutta we have not infrequently come across this organism in human faeces, and have several times cultivated it in Row's hæmoglobin-saline medium. It corresponds morphologically with *Tricercomonas intestinalis* Wenyon and O'Connor (1917). We have not so far seen the cysts of this organism.

It will be seen, therefore, that the generic name *Enteromonas* is now well established in the literature.

The genus *Tricercomonas* was founded by Wenyon and O'Connor (1917) for a flagellate found in human faeces having the general structure of a member of the genus *Cercomonas*, but possessing three anterior flagella instead of one. The fourth posterior flagellum was more or less attached to the surface of the body as it trailed backwards. The same organism has since been recorded by Kofoid, Kornhauser and Plate (1919) and Kofoid (1920) in soldiers who had returned to New York from service abroad. Lynch (1922) and Boeck (1924) have seen it in North America, Jepps (1923) in Malaya, and Da Cunha and Pacheco (1923) in Brazil. Boeck (1924) and J. G. Thomson and Robertson (1925) have cultivated this organism in Boeck's L, E, S medium.

hairs The inner occipital hair is long, thin and unbranched, a feature very characteristic of this species Less frequently this hair may be bifid or even trifid close to the apex, but such instances are rare Generally, the inner occipital hair is long and unbranched, and this character by itself would serve to distinguish this species from the others Even in those instances in which this hair is bifid, the branching is at some distance from the base of the hair and are easily recognizable from those of *A pallidus* or *philippinensis*, in both of which the first branching is close to the base (Plate XLVIII, B<sub>7</sub>—B<sub>8</sub> and C<sub>8</sub> to C<sub>7</sub>)

The outer occipital hair has 4 to 6 pinnate branches, occasionally more

*Shoulder-hairs*—Both the inner and median shoulder-hairs of *A fuliginosus* larva are strongly pigmented and possess well developed roots which coalesce in the mature larva (Plate XLV, fig 4) The inner shoulder-hair has a varying number of branches, ranging from 15 to 23, but in a large proportion of cases, the branches number 18 to 20 The median shoulder-hair has 13 to 16 branches

*Palmate hairs*—The thorax has a small palmate hair with 6 to 9 leaflets which have no filament The number of blades is occasionally more The first abdominal segment has a pair of small palmate hairs with 8 to 11 leaflets in each, the leaflets have no filament

The palmate hair on the 2nd abdominal segment is well developed and has 14 to 16 leaflets which are differentiated into a blade and a filament On segments 3 to 6, the palmate hairs generally have 16 to 18 leaflets, sometimes more On the 7th segment, the palmate hair has a fewer number of blades (14 to 16)

The palmate hair on the 5th abdominal segment is taken as the standard for comparison of the shape of the palmate hair leaflets with the other species The palmate hair of *A fuliginosus* has leaflets which are generally narrow (Plate XLV, fig 5), but may frequently be much broader (Plate XLV, fig 6) The filament is invariably long and thin The leaflets of this species are free from pigment and can be distinguished from *A pallidus* in which the leaflets are conspicuously pigmented

#### LARVA OF *Anopheles pallidus* THEOBALD

*General colouration*—Dull green to yellowish brown in colour, head brown White patches are ordinarily present on the anterior edge of the thorax and on the 8th abdominal segment, as in *A fuliginosus* Besides these, yellow spots are occasionally present on the 2nd, 3rd and 5th abdominal segments These latter spots may frequently be wanting

*Clypeal hairs*—Internal clypeal hairs with numerous branches along its whole length, more aggregated towards the tip, branches moderately long, thick and occasionally bifurcating at tip The branch hairs are thicker than those of *A fuliginosus* and more numerous (Plate XLVI, fig 1) External clypeal hairs are well pigmented, thick and dark-brown in colour The branching is compound, the main stem dividing into two or three main branches which again divide into smaller branches These branches are much thicker than those of *A fuliginosus*

flagellates With regard to the human species, we do not agree with this for a moment We have studied several strains of this organism, both in the fresh state as seen in human faeces and in culture, and it has a definite morphology of its own Wenyon (1926, p 636) points out that the form in termites which Duboscq and Grassé considered to be of *Tricercomonas* type possessed both an axostyle and a parabasal body, in which respects it differed from *T intestinalis* of man

On a due consideration of the literature, it seems to us perhaps best to adhere to Wenyon's view that the two genera should be differentiated from each other (provisionally at least)

If so, then the organism which we have discovered in the pig corresponds very closely in its general morphology with the genus *Tricercomonas* Wenyon and O'Connor (1917) It differs from *Tricercomonas intestinalis* Wenyon and O'Connor (1917), in its enormous size and in its sluggish motility, and is clearly a different species of the same genus

We suggest for it therefore the name *Tricercomonas suis*, n sp

We would direct the attention of laboratory workers and protozoologists to this organism It is a large parasite, easily cultivated, and very suitable for study A study of it may help to clear up the confusion which at present exists with regard to the genera *Enteromonas* and *Tricercomonas*

#### SUMMARY

A new species of *Tricercomonas* from the pig—*Tricercomonas suis*, n sp—is described It differs from the species of *Tricercomonas* previously described chiefly in its relatively enormous size and its sluggish motility It can be readily cultivated *in vitro*

(REFERENCES—No List of References was submitted by the Authors—ED)

out of a collection of larvæ, even by a naked eye examination. The head pattern is similar to *A. fuliginosus*, but the dark areas are more extensive.

*Clypeal hairs*—The median clypeal hairs are pigmented, densely branched and the branches are as thick as in *A. pallidus*. The basal half of the hair does not carry many branches, while the apical portion is quite thick with them. Some of the branches often divide into smaller branches.

The external clypeal hairs are very similar in appearance to the external clypeal hairs of *A. sinensis* or *barbivostris* (Plate XLVII, figs 1 and 2). The branching of this hair is complex, the main stem divided close to the base into several secondary branches which divide into smaller branches, which again divide into smaller ones. The result is a bunch-like hair, the branches of which are not all disposed in one plane. The external clypeal hairs are well pigmented and usually have more than 25 branches, and often as many as 40 or even more, but it is difficult to count the exact number of the branches on account of the crowding together of all the branches and their superposition over each other.

The posterior clypeal hair is characteristic in having 5 to 8, and sometimes more, palmate branches, the common form having 7. All the branches are of nearly equal length and start from nearly the same point close to the base of the hair and spread out in a palmate manner (Plate XLVIII, C<sub>2</sub> to C<sub>4</sub>).

*Occipital hairs*—The occipital hairs are inserted very nearly in one line, and although the outer occipital hairs start somewhat anteriorly to the bases of the inner occipital hairs, the difference is not as noticeable as in the case of *A. fuliginosus*.

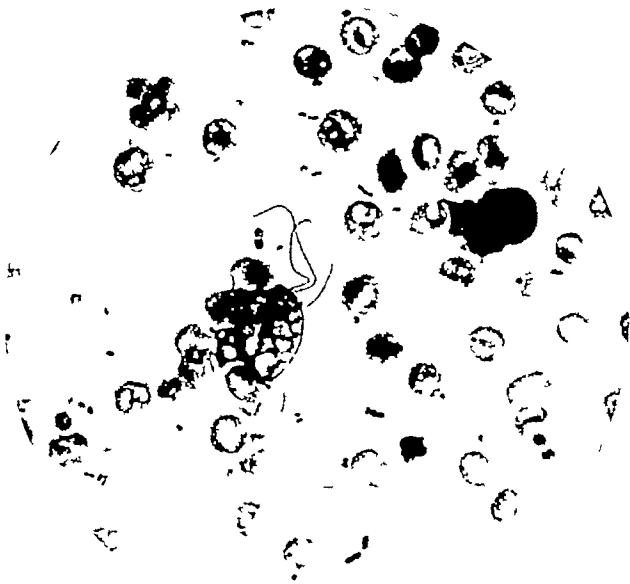
The inner clypeal hairs branch very close to the base of the hair and there are two to four dichotomous branches, the common form has 3 branches. The variations of this hair are figured in Plate XLVIII, C<sub>5</sub> to C<sub>7</sub>. The external occipital hairs are pinnately branched with 3 to 5 branches, occasionally 6 or 7 branches.

*Shoulder-hairs*—The internal and median shoulder-hairs have well developed roots which are distinct even in the mature larvæ and have never been observed to coalesce as in the two other species. The hairs are not pigmented. The internal shoulder-hair usually has 18 to 20 branches and the median shoulder-hair has 10 to 13 branches.

*Palmate hairs*—A small palmate hair with 9 to 11 leaflets is present on the thorax, the leaflets are long and pointed, and the tips of the leaflets are dark and pigmented. In the 1st abdominal segment occurs a small palmate hair with 9 to 11 leaflets. These palmate hair leaflets have no filaments. The 2nd abdominal segment has palmate hairs which have 12 to 15 leaflets with well developed filaments. On segments 3 to 6, the palmate hairs have 16 to 18 leaflets each, rarely more, while on the 7th abdominal segment, the palmate hair has 13 to 14 leaflets.

*Fifth abdominal palmate hair*—The blade of the leaflet is broad and terminates at an angle into a very thin and comparatively short filament (Plate XLVII, figs 3 and 4). The filament is less than double the breadth of the blade,

PLATE L



Showing very large size of *Tricercomonas*, also red blood corpuscles of the rabbit present in the same field

From the above, a synoptic table of the larvæ of the three species may be worked out as follows —

Inner occipital hair of larva simple (rarely branched close to apex), branches of external clypeal hairs start from main rachis, not thick or pigmented, 16 in number or less, palmate hairs without any distinct pigment

*A fuliginosus*

Inner occipital hair of larva branched close to base, branches of external clypeal hairs starting from the main rachis as also from secondary branches, more than 16 in number, thick and pigmented, palmate hairs distinctly pigmented

Conspicuous white spots on segments 2, 5 and 6, roots of shoulder-hairs not fused, posterior clypeal hair with 5 or more equally long palmate branches, palmate hair leaflet with broad blade and short and thin filament

*A philippinensis*

No conspicuous spots on body, roots of shoulder-hairs coalescing in mature larva, posterior clypeal hair with less than 5 branches which are pinnate, palmate hair leaflet with a narrow blade and long and thick filament

*A pallidus*

The table of differences and the synoptic table given above of the larvæ have been of great help to the writer in the identification of larvæ from different parts of Bengal. Their reliability is borne by the fact that the larval identifications have always been verified by an examination of the adult which bred out of the specimen. Much of the value of previous records by larval identification of *A fuliginosus* or of the two other species is lost because of the possibility of the species being mistaken one for the other. The proper identification of specimens belonging to these species is of more than academic interest, as it appears likely that the three species vary appreciably in regard to their susceptibility to malarial infection and their capacity to transmit the parasites.

The writer is much indebted to Dr Panchanan Sur, M B, D P H, Assistant Surgeon, Bengal Public Health Department, for considerable help received from him by way of specimens.

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hours later a moderate growth of the organism was obtained in this culture, but unfortunately a coprozoic *Bodo* had also appeared, and also a *Blastocystis*. At forty-eight hours' growth the very heavy growth of *Bodo* and of *Blastocystis* killed out the *Trichomonas*. The material at our disposal was, therefore, very scanty.

Films were prepared and stained by the method advocated by Shortt (1923) for leishmania, which we have found to be an excellent and rapid method for staining the vegetative forms of intestinal flagellate protozoa. (The essential steps of Shortt's method are fixation of the fresh material over osmic acid, then drying as rapidly as possible under a fan, and staining with Giemsa's stain.)

As seen under the dark ground microscope, the organism is a typical *Trichomonas*, possessing three anterior flagella, a very well developed axostyle, and a strongly developed undulating membrane, from the end of which the terminal portion of the fourth trailing flagellum projects free.

We are much indebted to Mr J K Mullick, artist to the department, for the colour plate which illustrates this *Trichomonas*, as seen in films stained by Shortt's method (see Plate LI).

We were able to make measurements on only 15 individuals. These were drawn with the camera lucida, the stage micrometer scale was then drawn alongside them, dividers set to 2  $\mu$  on this scale, and the different dimensions stepped off with the dividers. The results were as follows —

	Length of body	Greatest width of body	Anterior flagella	Posterior flagellum
Smallest observed	18 $\mu$	6 $\mu$	8 $\mu$	24 $\mu$
Mean of 15 observations	21.9 $\mu$	8.95 $\mu$	15.4 $\mu$	32.2 $\mu$
Largest observed	26 $\mu$	14 $\mu$	25 $\mu$	45 $\mu$

(The measurements for length of body are taken from the anterior pole along the curved line of the axostyle to the tip of the posterior pole. The length of the posterior flagellum was stepped out from the basal granule along the margin of the undulating membrane and finally along the terminal free portion of the flagellum to its end.)

No cyst of the organism was observed, either in the fresh caecal contents or in the culture.

Wenyon (1926, p. 660) points out that at present the classification of species of the genus *Trichomonas* is in a confused state. 'Species of *Trichomonas* are very common parasites of the intestinal canals of animals,' he writes. 'The caecums and large intestines of guinea-pigs and rats, for instance, are often swarming with these flagellates, which, on account of their large size, are more easily studied than the human forms. They are common in birds, reptiles and



1



2



3



4



5



6



EXPLANATION OF PLATE XLVI

*Anopheles pallidus* Theobald (Photomicrographs)

- Fig 1 Clypeal hairs, internal, external and posterior  
„ 2 Outer occipital hairs of the left side The inner occipital hair (on  
right) is dichotomously branched  
„ 3 Shoulder-hairs (left side)  
„ 4 Posterior region of abdomen of larva skin showing palmate hairs and  
scutæ (segments 2 to 7)  
Figs 5 and 6 Palmate hairs showing pigmentation

# SOME PATHOLOGICAL ASPECTS OF CHRONIC APPENDICITIS

## I THE LYMPHOID TISSUE OF THE APPENDIX

BY

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[Received for publication, August 11, 1928]

THE vermiform appendix has been compared to a Peyer's patch which has been pulled out from the lumen of the gut. The importance of this lymphoid tissue is evident when one has to consider disease of the appendix from a pathological stand-point. This lymphoid tissue is normally aggregated into follicles with well defined germ centres. There is a thin muscularis mucosa which separates the follicles from the submucous coat and rises up in between the follicles towards the mucous membrane. Apart from these follicles there is a certain amount of loose lymphoid tissue in between the follicles so that if one examines a series of cross-sections of a normal appendix, one is struck with the number of lymphoid cells that are present in the submucous coat as well as in between the vessels, sometimes forming definite perivascular zones such as one would associate with an inflammatory process. It is, however, evident that in such an essentially lymphoid organ, the presence of lymphoid cells in the deeper layers does not have any special significance.

It has been universally regarded that the lymphoid tissue is most abundant in childhood and that it gradually decreases with age until in old age most of the lymphoid structures undergo an involutary fibrosis such as one meets with in a senile spleen. Also it is equally true that a lymphoid hyperplasia may be present in conditions which go under the vague term of lymphatism. Lockwood estimates that there are about 300 lymphoid follicles in a normal appendix about  $3\frac{1}{2}$  inches long.



TABLE I

*The lymphoid follicles in a normal appendix*

Number of normal appendices examined	Average number of follicles in each section	Average number of germ centres	Largest number of follicles in a section	Largest number of germ centres in a section
198	6.45	3.27	15	15

TABLE II

*Age variations of the lymphoid tissue in the normal appendix*

Age.	Number of follicles	Number of germ centres	Age.	Number of follicles	Number of germ centres
5	12.0	5.0	30	6.0	2.8
7	10.0	8.0	31	7.0	4.6
10	6.0	0.0	32	6.8	1.3
12	6.0	3.0	33	6	2.5
13			34	6.3	4.0
14	9.0	6.0	35	6.6	4.0
15	8.25	4.0	36	6.6	2.0
16	8.5	7.5	37	7.5	4.5
17	11.5	9.0	38	6.75	3.0
18	9.3	8.3	39	4	4
19	8.0	4.0	40	5.8	1.8
20	7.9	3.4	41		
21	8.5	5.5	42	5	0
22	6.75	2.5	43	3.5	0
23	4.75	3.6	44	.	.
24	6.89	3.1	45	6.7	3.4
25	7.58	3.5	46	..	..
26	6.0	3.0	47	.	
27	6.2	4.0	48	6.4	3.0
28	7.7	5.56	49		.
29	6.5	3.5	50	5.6	2.8

EXPLANATION OF PLATE XLVIII

A—*Anopheles fuliginosus* Giles

- A<sub>1</sub> Head of larva, × 98  
A<sub>2</sub> to A<sub>5</sub> Variations in posterior clypeal hair, × 415.

B—*Anopheles pallidus* Theobald

- B<sub>1</sub> Head of larva, × 98  
B<sub>2</sub> to B<sub>4</sub> Variations in posterior clypeal hair, × 415  
B<sub>5</sub> to B<sub>6</sub> Variations in inner occipital hair, × 415

C—*Anopheles philippinensis* Ludlow.

- C<sub>1</sub>. Head of larva, × 98  
C<sub>2</sub> to C<sub>4</sub> Variations in posterior clypeal hair, × 415  
C<sub>5</sub> to C<sub>7</sub> Variations in inner clypeal hair, × 205  
C<sub>8</sub> Larva as seen in reflected light, showing white spots on body, × 20  
(All camera lucida drawings)



SUMMARY

(1) A cross-section of a normal appendix shows on an average about 6 lymph follicles and 3 well defined germ centres

(2) In a chronically inflamed organ there is a tendency for atrophy of the lymphoid tissue to occur

(3) Lymphoid cells are normally found in numbers in the submucous coat of the appendix

(4) There is not any marked involuntary atrophy of the lymphoid tissue of the appendix with age between the ages 10 and 50

(5) There seems to be a definite relationship between lymphoid hyperplasia of the appendix in children as well as adults and clinical symptoms of colicky pain in the lower abdomen

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# A *TRICERCOMONAS* OF THE PIG

BY

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AND

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ON the 16th May, 1928, Dr P A Maplestone, D S O, Hookworm Research Worker, Calcutta School of Tropical Medicine, had occasion to kill an experimental pig which had been fed on hookworm larvæ. Before doing so, he very kindly informed us that this material would be available in case we wanted material for demonstration of *Balantidium coli*, *Balantidium suis*, or other intestinal protozoa to students.

Arrangements for a complete autopsy were made accordingly, and the carcass was received in the Protozoology Department in a perfectly fresh condition.

No protozoa or other blood-inhabiting parasites could be found in thin and thick blood films taken from the animal's cardiac blood. No *balantidia* were found in the contents of the colon. When we came to examine the contents of the cæcum, however, we found large numbers of a large and sluggishly motile flagellate protozoon. At first sight under the dark ground this organism appeared to be an unusually sluggish *Trichomonas*, but with the undulating membrane very badly defined. Closer inspection, however, showed that there was no undulating membrane at all, but that what gave rise to the appearance resembling the ripple of an undulating membrane was the beating of a prominent fourth, posterior, trailing flagellum which arose from the anterior pole of the animal, passed back along the surface of the body, and projected free in a postero-lateral direction. On examination of films fixed and stained by the method advocated by Shortt (1923) for leishmania, the true character of the flagellate became apparent, and it appears to belong to the genus *Tricercomonas*.

between the lymphoid cells that lie below the short glands that usually occur in the appendix. They should on no account be confused with the larger and uniformly staining oxyntic cells that occur in the gastric mucous membrane and which no doubt take the eosin stain.

TABLE I

*Eosinophile infiltration of the mucosa of the appendix*

Number of normal appendices examined	Number of cases in which eosinophile infiltrations were found	Percentage
193	183	94.8
Number of diseased appendices examined	Number of cases in which eosinophile infiltrations were found in the mucosa	Percentage
126	112	88.9

Table I shows the extreme frequency with which these cells occur in the normal appendix as well as in chronic appendicitis. The question may, therefore, be raised whether eosinophilia of the mucosa of the appendix is a normal phenomenon at least in the tropics.

In chronic appendicitis again one finds these cells in the mucous membrane in a large proportion of cases, but here the eosinophile cells are not only found in the mucous membrane but have wandered in numbers to the submucosa which, in many cases, shows well marked features of chronic appendicitis such as thickening, fibrosis, endarteritis of the vessels, increase of fat, etc., indeed in many cases of chronic appendicitis the cells have infiltrated into the muscular coat. Table II shows the high percentage of submucous eosinophilia in chronic appendicitis.

TABLE II

*Eosinophile infiltration of the submucosa of the appendix*

Number of normal appendices examined	Number showing submucous eosinophilia.	Percentage
198	19	10.04
Number of chronic appendices examined	Number showing submucous eosinophilia.	Percentage.
132	117	88.6

*Note*—Well marked eosinophilia was found in 18 cases while in 42 cases the eosinophilia was moderate, and in 57 cases only a few eosinophiles were found.

Mr J K Mullick, artist to the department, for the coloured plate which accompanies this article, and which was drawn with the Abbé camera lucida. The photomicrograph is taken from a preparation stained by Shortt's method from a culture in Row's medium (Plate L). It shows the very large size of this *Tricercomonas*, as compared with the red blood corpuscles of the rabbit which are also present in the same field. The three anterior flagella are seen projecting from the front pole of the parasite, and the fourth flagellum projecting posteriorly.

The parasite is not as amoeboid as is indicated in the plate, the distorted appearance is probably due to the method of fixation employed (fixation over osmic acid, and then with methyl alcohol). There is a single large nucleus of vesicular pattern near the anterior pole. The arrangement of the flagella is well seen in Plate XLIX, figs 1, 3, 4, 5, 6, 7, 8, 18 and 19. The parasite is a voracious feeder, and freely ingests bacteria, etc. We have not been able to make out the existence of a cytostome, however, in either fresh or stained preparations, and feeding seems to be accomplished by ingestion of bacteria, etc., through the surface pellicle—probably by pseudopodial action, as is the case with *Cercomonas longicauda*.

With regard to the size of the parasite, 50 specimens were drawn with the camera lucida from films stained by Shortt's method. The stage micrometer scale was then drawn alongside, dividers set to  $2\ \mu$  on the scale, and the different dimensions stepped off in microns with the dividers. The results were as follows —

	Length of body	Greatest breadth of body	Anterior flagella	Posterior flagellum
Smallest observed	$9\ \mu$	$6\ \mu$	$8\ \mu$	$9\ \mu$
Mean of 50 observations	$15.9\ \mu$	$11.8\ \mu$	$14.1\ \mu$	$17.3\ \mu$
Largest observed	$23\ \mu$	$14\ \mu$	$18\ \mu$	$26\ \mu$

As there is apt to be a little shrinkage on fixation by osmic acid in Shortt's method, the actual size of the living flagellate is probably a little larger than the means given.

No cyst was observed in the caecal contents of the pig, nor has any been formed so far in any of the cultures put up.

#### DISCUSSION

There has been considerable discussion in the literature with regard to the genera *Enteromonas* and *Tricercomonas*.

The genus *Enteromonas* Da Fonesca (1915), was founded by Da Fonesca for a small flagellate protozoon, named by him *Enteromonas hominis*, which he found in human faeces in Brazil. Subsequently (Da Fonesca, 1916, 1918, 1920) he published several re-descriptions of this parasite, which are not in agreement



Now the muddle in the literature arises from the fact that different workers have described what is apparently one and the same organism from human faeces alternatively as *Enteromonas hominis* and *Tricercomonas intestinalis*. The question at issue is as to which generic name should stand. Dobell and O'Connor (1921) come to the conclusion that *Enteromonas* has priority, and give the following description of *Enteromonas hominis*.

'*Enteromonas hominis* is a very small oval or round flagellate, of somewhat changeable shape, measuring usually  $4\ \mu$  to  $8\ \mu$  in length when alive. Stained specimens measure somewhat less. The flagellate possesses a single vesicular nucleus, situated at the anterior end and containing a large central karyosome. The nucleus is more or less drawn out at its anterior pole, and at this point there are at least two (probably more) minute blepharoplasts, which give origin to the four flagella. These are approximately equal in length. Three of them are free, and are directed forwards, the fourth, which may be slightly longer, is directed backwards. It passes over the surface of the body, to which it is adherent (? always), and terminates freely at the hind end, or sometimes laterally. The cytoplasm contains food vacuoles, enclosing ingested bacteria. There is no permanent mouth, however, and no axostyle, no undulating membrane, or other conspicuous organ.' Dobell and O'Connor then proceed to describe the cyst of '*Tricercomonas intestinalis*,' taking their account from Wenyon and O'Connor (1917), and attribute these cysts to the genus *Enteromonas*.

This procedure has at least the merit of simplicity, and in his 'Introduction to Medical Protozoology' (1928), the senior author of this paper followed Dobell and O'Connor's classification, as it seemed to him to be the simplest one. J. G. Thomson and Robertson (1925) however refuse to accept Dobell and O'Connor's contention that *Enteromonas* and *Tricercomonas* are identical, and prefer to differentiate between the two. They give a good account of the cysts of the latter.

Wenyon (1926, p. 307) considers (a) that Da Fonesca's *Enteromonas hominis* is probably a different organism from *Tricercomonas intestinalis*. It possesses only three flagella, whereas the latter has four. Further, *Enteromonas* has a much more rounded body than *Tricercomonas*.

(b) That *E. hominis* of Chalmers and Pekkola were merely small rounded forms of *Chilomastix mesnili* with obscured rounded cytostomal groove, and that possibly Da Fonesca made a similar mistake.

(c) That Da Fonesca's *E. intestinalis* of the rabbit is possibly only a small rounded form of *Chilomastix cuculi* of the rabbit.

(d) That, for the time being, it is better to retain *Enteromonas* and *Tricercomonas* as separate genera, but that further study may show that '*Enteromonas*' is only a small rounded form of *Chilomastix*, in which case *Enteromonas* becomes synonymous with *Chilomastix*, and the name should disappear from the textbooks.

The case is still further complicated by the studies of Duboscq and Grassé (1924) on the flagellates of termites. These workers come to the conclusion that both *Enteromonas* and *Tricercomonas* are merely young forms of other

# AN INVESTIGATION INTO THE CAUSE OF ASCITES

BY

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AND

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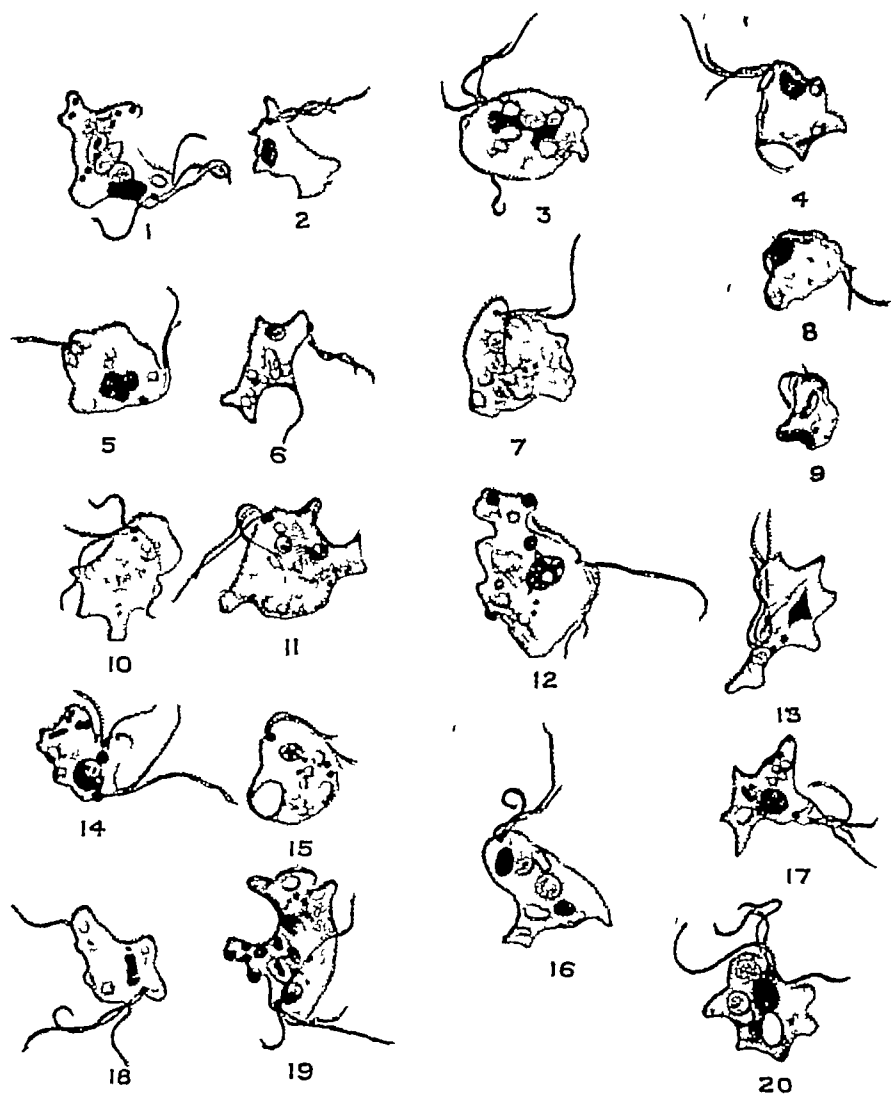
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*(Under the auspices of Indian Research Fund Association)*

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THE cause of ascites in a certain number of cases that are admitted into the hospitals in India appears to be obscure. In places where there is no laboratory facility for investigation, these cases are usually diagnosed as hepatic, renal, cardiac or tuberculous in origin. We notice amongst the admissions in the Patna General Hospital a group of ascites cases, the origin of which can not be satisfactorily explained from clinical findings. Similar obscure cases(1) have been observed in European countries where the probable cause of the condition has been attributed to abdominal tuberculosis or to 'proliferative peritonitis'. The views put forward on such cases by observers in India are conflicting. Cunningham(2) in 1917 remarked from his personal observation in Eastern Bengal on the occurrence of ascites cases with cirrhosis of the liver as a complication of dysentery. Rogers is said to have drawn attention to this relationship sometime before. Megaw(3) in 1921 reported a number of ascites cases from Lucknow which he ascribed to chronic peritonitis of dysenteric origin, and again(4) in 1924 from Calcutta confirming his previous conclusion. The latter observer has brought forward the result of the agglutination reaction in these cases together with certain post-mortem findings in support of his views. Sprawson(5) in Lucknow designated the disease as 'chronic superior peritonitis'. No further report is available on the occurrence of such cases in other parts of India. It may be regarded, therefore, that a number of cases of ascites which are clinically obscure and which have been associated with dysentery by more than one observer, are common in Bengal, Behar and the United Provinces.

# PLATE XLIX



0 10 20 30 40 50  
SCALE



*Course of the disease*—When first seen the patient has a shrunken face, emaciated limbs, an enlarged abdomen with a varying amount of fluid, dryness and loss of elasticity of the skin. The complexion is appreciably altered. Anæmia is not a marked feature. There is no evidence of jaundice. The veins of the anterior abdominal wall are not prominent except in extreme cases. The 'Caput medusæ,' hæmorrhoids, hæmatemesis and melæna are absent. Oral sepsis is common. The disease, as a rule, runs an afebrile course.

When the ascites is marked, cedema of feet and legs supervenes, the veins of the anterior abdominal wall become prominent, and the diaphragm is pushed up causing respiratory and cardiac embarrassment, the total urinary output is diminished and there is occasionally a trace of albumen in the urine. All these symptoms rapidly disappear when the fluid is withdrawn by paracentesis, thus showing their mechanical origin. The fluid, however, accumulates sooner or later.

Various forms of medical treatment have been given without any success. To render temporary relief we have resorted to repeated tapplings which the patients stand very well. As all patients leave hospital with a varying amount of improvement, it has not been possible to trace the mode of termination. There were no deaths in the hospital.

#### INVESTIGATION

With a view to throwing some light, if possible, on the basic cause underlying the above syndrome, we have carried out investigations into the efficiency of the renal, cardiac and hepatic systems and have also made a detailed study of the condition of the blood and ascitic fluid in these cases. The results of which we now propose to put forward.

#### *Renal efficiency tests*

Routine chemical, microscopic and cultural examination of the urine, revealed nothing except a trace of albumen in Case XIV. Table I shows the results of the chloride estimation and urea concentration test. We realize that excretion of chloride *per se* without reference to diet is of no significance, but estimation of chloride in the urine collected at all times of a large number of mixed cases on an average Indian diet shows that it varies from 0.6 to 0.8 per cent. The samples of urine for chloride estimation in our series was collected 2 to 3 hours after an ordinary hospital diet.

For the urea concentration test(6), 15 grms of urea in 100 ccs of water were given on an empty stomach. The samples of urine were collected just before urea was given, and 1 and 2 hours afterwards.

Out of 19 cases, only 4 showed renal inefficiency to both urea and chloride excretion.

From the absence of any abnormal constituents in the urine and from the results of the two tests, it would appear that in majority of the cases the renal function is effective.

# A NOTE ON A *TRICHOMONAS* OF THE PORCUPINE

BY

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THE history of a porcupine may be of interest, since it is a somewhat unusual laboratory animal.

In connection with a criminal case in the courts, a statement was made that a certain blood stain on some earth was due to porcupine blood. Lieut-Col. R. B. Lloyd, I M S., Imperial Serologist to the Government of India, accordingly set about preparing anti-porcupine serum. A porcupine was obtained and imported into the Calcutta School of Tropical Medicine and Hygiene. He travelled up to the Imperial Serologist's department in the electric lift in company with several clerks and laboratory assistants, and seemed decidedly uncomfortable in the lift (Presumably he is the first porcupine to have travelled in an electric lift). In the Serology Department the animal was anaesthetized and bled to death from the jugular vein. Colonel Lloyd then very kindly made over the perfectly fresh carcass to the Protozoology Department of the School, as it was considered that the opportunity of examining so unusual a laboratory animal for parasitic protozoa should not be missed.

Thin and thick blood films from the heart blood showed no blood-inhabiting protozoa or other parasites. In the contents of the cæcum, however, there were found very scanty numbers of a typical *Trichomonas* in its motile, vegetative phase.

A culture was immediately taken in Row's hæmoglobin-saline medium (Row, 1914, Knowles, 1928, p. 653) and placed in the 37°C incubator. Twenty-four

*Hepatic efficiency*

We applied two functional tests, namely, the 'lævulose tolerance' and Widal's hæmoclastic crisis. The majority of observers agree that these methods are reliable and yield valuable information. Shirokauer(7) noted that there is little or no rise in the blood-sugar content in a normal person one hour after the administration of 100 grms of lævulose. If, however, the liver is damaged, the rise is marked. The dose of lævulose in our series varied with the patient's body weight as recommended by Spence and Brett(8)

A weight of 40 kg or 40 seers received 30 grms

„ 50 „ 50 „ „ 35 „

„ 60 „ 60 „ „ 40 „

Widal(9) demonstrated that a post-prandial leucocytosis, which usually comes to +3,000 per c mm at the end of 20 to 40 minutes in healthy individuals, depends on the functional integrity of the liver, and, in cases where this organ was diseased, the leucocytosis is not appreciable. In some cases there is actual leucopenia.

We have verified this test on some normal individuals as well as on known liver diseases and consider it fairly reliable. The lævulose test, however, is more delicate.

In Table II, the lævulose tolerance test shows that of the 19 cases, 16 had slightly or definitely deranged function. Out of 8 cases with enlargement of liver, 7 had deranged function and out of 11 cases without an enlarged liver, 9 had deranged function.

It appears, therefore, that a liver which is not enlarged and which does not show any clinical evidence of disease may be functionally defective.

Widal's hæmoclastic crisis shows that out of 15 cases 11 showed deranged hepatic function.

These two tests, although slightly different in their respective results, agree on the main issue as to the efficiency of the liver. Taken conjointly, then, it may be concluded that the majority of the ascites cases suffered from impaired function of the liver.

*Blood examination*

Table III gives a cellular and serological study of blood.

Eleven cases out of 16 had a total W B C count of less than 6,000 per c mm. The total W B C count of 20 healthy individuals of the cultivator class showed an average of about 7,000 per c mm. The majority of the cases had, therefore, a slight leucopenia, except Cases VII and XV, which showed a slight degree of leucocytosis. Case VII had a marked increase in the polymorphonuclear cells. In this case the ascitic fluid proved to be an exudate and not a transudate, as it proved to be in the remainder of the cases. Four cases showed an eosinophilia, of which 3 had ankylostomiasis and 1 bronchial asthma. There was no appreciable increase in the large mononuclear cells in any case. All blood slides were negative to malaria. The hæmoglobin content was slightly below normal.

The degree of enlargement of the spleen was estimated and in 5 cases out of 16 varied from being palpable to extending to 3 inches below costal margin.

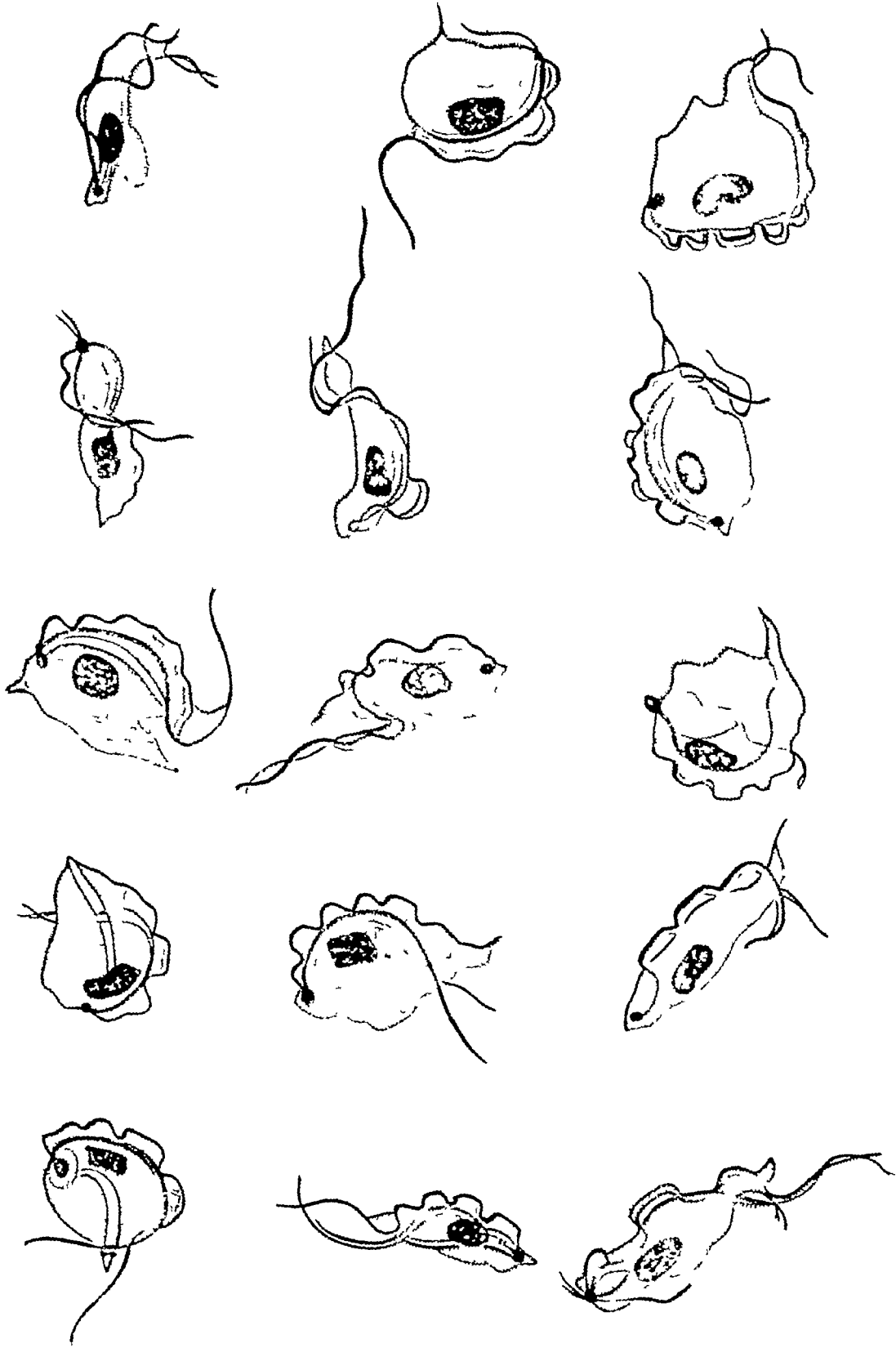


TABLE III

*Blood examination*

Case No	Spleen	Total W B C per c.c.s	DIFFERENTIAL COUNT				Hæmoglobin Per cent	Formol-gel test	Urea-stibamine test	Wassermann action	Calcium content in mg per 100 c.c
			Polymorphs Per cent	Lymphocytes Per cent	L, M Per cent	Eosinophiles Per cent					
V	+	4,688	64	28	5	3	65	+++	+++	—	
IV	+	5,936	69	22	5	4	65	—	—	—	87
VI	+	5,000	68	10	4	17	70	+++	+++	—	92
VII	+	7,500	90	8	1	1	70	—	—	—	88
VIII	+	4,800	66	28	5	1	75	+++	+++	—	95
IX	—	6,250	75	22	3	0	70	—	—	—	106
X	—	4,062	79	16	4	1	65	—	—	—	81
XI	—	4,687	74	23	3	2	75	—	—	—	92
XII	—	3,900	66	18	4	2	65	—	—	—	89
XIII	—	4,687	74	22	2	2	68	—	—	—	92
XIV	—	4,800	68	20	4	8	65	—	—	—	93
XV	—	9,062	39	25	4	32	45	—	—	—	90
XVI	—	6,250	63	30	6	1	55	+++	+++	+++	70
XVII	—	5,000	68	21	6	5	55	++	++	—	96
XVIII	—	6,250	58	28	6	10	55	—	—	—	98
XIX	—	5,937	56	38	2	4	65	—	—	—	96

+ = Spleen enlarged

— = Spleen not enlarged

— = Formolgel, Urea-stibamine or Wassermann tests negative

+, ++, +++ = Tests positive in varying degrees

amphibia, and also occur in invertebrates. Many of these have been given distinctive names, but whether each host has its own species cannot be stated at present. The various species are very uniform in character, and differ from one another chiefly in size. *T. muris* of the mouse varies in length from 3 to 20 microns at least, so that dimensions are of little value as specific characters unless they can be proved to be constant.

For this reason we have resisted the temptation to give this parasite a specific name. It corresponds very closely in size and general morphology with *Trichomonas muris* (Grassi, 1879) and also with *Trichomonas caviae* (Davaigne, 1875). The porcupine is a rodent, and it may well be that the *Trichomonas* of rodents may all be one and the same organism. The Indian porcupine (*Hystrix bengalensis*), however, must now be added to the already long list of hosts which harbour a natural *Trichomonas* of their own.

We are much indebted to Colonel Lloyd for supplying us with such interesting material.

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*paratyphosus* A and *paratyphosus* B in the blood. Four cases were positive to *B typhosus* up to a 1 in 40 dilution, 4 cases to *B paratyphosus* A, up to 1 in 10 and 1 case to *B paratyphosus* B to 1 in 10 dilution. A positive reaction in such low dilutions does not appear to be of much import.

Sixteen cases out of 19 showed a positive reaction to dysentery organisms of the Flexner type, 6 were positive up to 1 in 80, seven to 1 in 40 and three to 1 in 20. Four out of the above 16 cases showed a positive reaction to *B shiga* as well, three up to 1 in 20 and 1 to 1 in 40. *B dysenteriae* (Flexner) was isolated from the stools in one case only. The majority of the cases thus showed previous evidence of an infection by the desenteric organisms.

### Examination of the ascitic fluid

Table V shows the nature of the ascitic fluid. In 14 cases out of 15, the specific gravity was low, the fluid was clear and did not clot on standing, thus

TABLE V  
*Examination of the ascitic fluid*

Case No	Sp gr	Consistence	Clotting	Runeberg test	Cells per c mm	Effect on inoculated guinea pigs
V	1011	Clear	Nil	Transudate	135	No effect on guinea pigs
VI	1011	"	"	"	150	" "
VII	1019	"	Present	Exudate	165	Animal died of peritonitis in 24 hours, experiment repeated with same result
VIII	1011	"	Nil	Transudate	70	No effect.
IX	1008	"	"	"	90	Died next morning, no lesion
X	1009	"	"	Doubtful		No effect
XI	1008	"	"	"	105	"
XII	1009	"	"	Transudate	150	,
XIII	1007	"	"	"	135	"
XIV	1012	"	"	"	150	,
XV	1009	"	"	"	120	"
XVI	1012	"	"	"	165	Died next morning, no lesion
XVII	1011	"	"	"	135	No effect
XVIII	1011	"	"	"	105	"
XIX	1013	"	"	"	135	"

A cross-section of a normal appendix when examined under the microscope shows on an average about 6 follicles and about 3 well defined germ centres

Table I represents the findings in a series of cross-sections of different appendices which have been histologically reported as normal

It is also of interest to correlate the variations in the lymphoid tissue with age Table II represents the age variations in a series of normal cases

It is at once apparent that between the ages of 10 and 50, there is only a little of the involution atrophy that is said to occur with age The only evidence of marked increase of lymphoid tissue is found below the age of ten where follicle counts of 12 and 13 with a corresponding number of germ centres, can be made out It should be understood that each of these figures represents the average of a series of cases of the same age

When we take into account the lymphoid tissue in cases of chronic appendicitis there is a great variation depending on the types of cases, but, on an average, the tendency is for the lymphoid tissue to atrophy and the germ centres to become less defined A comparison of the process underlying a chronic inflammation in a lymph gland shows the same atrophy of the lymphoid tissue and the germ centres with a corresponding fibrosis of the glandular structure Table III gives a list of the series of chronically inflamed appendices in which the lymphoid tissue was ill-defined as well as the series in which the germ centres were absent In 4 cases of chronic appendicitis, the lymphoid tissue was absent These were not, however, cases of obliterative fibrosis of the organ in which we meet with almost a complete absence of lymphoid structures

With regard to the lymphoid hyperplasia of the appendix and its clinical association with appendicitis, to which attention has been drawn by Smith, the series of cases examined (Table IV) shows the well defined relationship which is equally marked in young adults as in childhood, a point to which attention has not hitherto been drawn Clinically some of the cases had irregular attacks of pain, sometimes referred to the upper abdomen, sometimes definitely referred to the right iliac fossa In others, a definite diagnosis of recurrent appendicitis was made and pathologically only a lymphoid hyperplasia could be made out in an otherwise normal appendix

When we come to consider why this lymphoid hyperplasia should produce symptoms of appendicular colic, it is conceivable that swelling of the lymph follicles as a result of a mild catarrh might produce distension of the organ and stretching of the peritoneal coat resulting in pain That pain, as a viscerosensory reflex could be produced by distension, has been pointed out by Clifford Allbutt in relation to the aorta This swelling of the lymph follicles and subsequent turgidity of the organ might be the result of a mild infection which does not leave behind it any trace, just as a mild sore-throat and catarrh of the tonsils produces pain in a patient with a tonsillar hyperplasia



toxin derived from the lesions in the bowels caused by the dysenteric organisms, producing various morbid changes and giving rise to ascites can, therefore, be accepted

Ascites with derangement of hepatic function in a large number of cases naturally suggests that cirrhosis of the liver is the main ætiological factor. The toxin absorbed from an infected bowel, and producing interstitial hepatitis, is a factor in the development of ascites which requires careful examination.

The hepatic efficiency tests produce some evidence in favour of the deranged function of the liver as a whole, but it is impossible to determine which portion of the liver is damaged. Diseases like cirrhosis, fatty degeneration and carcinoma of the liver produce a similar picture. Since the parenchymatous portion of the liver is mainly responsible for the functional integrity of the organ, it is our belief that the functional derangement is only observed when the parenchyma cells are affected. The crude interstitial tissue, which can suffer injuries much more than the parenchyma tissue, and which undergoes a varying amount of proliferation in cirrhosis of the liver, thus indirectly helping in the development of ascites, does not show its derangement by hepatic function tests unless there is also associated damage to the parenchyma cells. We notice deranged function of the liver shortly after intravenous injection of organic arsenic, or after chloroform anaesthesia, in such cases the parenchyma cells are damaged. On the other hand, marked cirrhosis of the liver may only show slight impairment of function by these tests. For example, a recent case of atrophic cirrhosis of the liver, which by biochemical tests showed only slight derangement of the hepatic function, was submitted to a Talma Morison operation and, on histological examination of a bit of liver tissue taken during the operation, showed an advanced degree of multi-lobular cirrhosis. Definite derangement of the hepatic function in a large number of our cases, therefore, tends to show that the liver cells have been acted upon by some toxin,—probably dysenteric in origin.

Since it is not possible to ascertain the presence or degree of damage to the interstitial tissue by biochemical tests, we have to look in other directions for information. There has been no post-mortem evidence in our series. The post-mortem evidence put forward by one observer, however, shows general fibrotic thickening of the peritoneum in the upper part of the abdomen and absence of intra-hepatic fibrosis. Further, the remarkable absence of the classical signs and symptoms, ability to withstand several tapplings for the relief of ascites, a comparatively longer duration of life than is usual in atrophic cirrhosis, in which the ascites is a late and terminal phenomenon, are points which weigh against cirrhosis of the liver as an ætiological factor.

The study of the ascitic fluid shows that in almost all the cases it was a transudate with a preponderance of endothelial cells. In one case only (Case VII, Table V) the fluid was an exudate and contained polymorphonuclear cells. The high percentage of endothelial cells in the effusion shows that it has a mechanical origin. This finding also excludes tuberculosis and acute or sub-acute infections. The tuberculous nature of the process can further be eliminated

TABLE III

*The lymphoid tissue in chronic appendicitis*

Number of appendices examined	Average number of follicles	Average number of germ centres
115	4.77	2.09

Number of appendices in which the lymphoid tissue is diffuse and not aggregated into follicles 29

Number of appendices in which the germ centres were ill-defined 12

Number of appendices in which lymphoid tissue was absent 4

TABLE IV

*Lymphoid hyperplasia and clinical symptoms*

Age	History	Number of follicles	Number of germ centres
23	Appendicitis	11	8
17	Pain in right iliac fossa	12	10
35	Abdominal pain	11	8
33	Recurrent appendicitis	13	12
24	Acute colic	10	
18	Abdominal pain	10	8
26	Pain in right iliac fossa	10	2
25	Irregular pain	12	8
30	Irregular pain	13	10
35	Irregular pain	10	10
21	Pain in lower abdomen	11	8
28	Pain in lower abdomen	11	8
25	Pain in lower abdomen	15	6
38	Pain in lower abdomen	10	5
16	Pain in right iliac fossa	11	16
18	Pain in right iliac fossa	14	15
21	Pain in right iliac fossa	13	10

3 The renal and cardiac functions are good in the majority of cases

4 There is evidence of impaired function of liver in 84 per cent of cases

5 The ascitic fluid is a transudate with a preponderance of endothelial cells  
It is not tuberculous in origin

6 Taking the cases with no history of kala-azar only, a definite relationship with bacillary dysentery is established. In the absence of sufficient evidence in favour of cirrhosis of the liver, tuberculosis and inflammation as ætiological factors, a mechanical origin of the fluid due to disturbance of the absorptive power of the upper part of the peritoneum caused by previous dysenteric infection, seems probable

#### ACKNOWLEDGMENTS

We are indebted to Lieut-Col H. R. Dutton, I M S, Superintendent, Patna General Hospital, for giving us every facility in the hospital in carrying out this investigation. Thanks are also due to our colleagues in the Pathology Department, Prince of Wales Medical College, Patna

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# SOME PATHOLOGICAL ASPECTS OF CHRONIC APPENDICITIS.

## II EOSINOPHILE INFILTRATION OF THE APPENDIX

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THE presence of the eosinophile leucocytes in relation to various tissues and organs has been the subject of much speculation. This tissue-eosinophilia has been observed to occur in the base of chronic ulcers of the stomach and generally in intestinal ulcerations. I have observed in tumours of the intestine such as myomata a number of eosinophile cells infiltrating the growth. Eosinophilia is a well-marked feature of the histological picture in Hodgkin's disease as pointed out by Reed. Longcope mentions them as occurring in large numbers in the lymph nodes in fatal cases of diphtheria and scarlet fever. A few eosinophiles may be met with in the glands in tuberculosis, metastatic carcinoma and sarcoma.

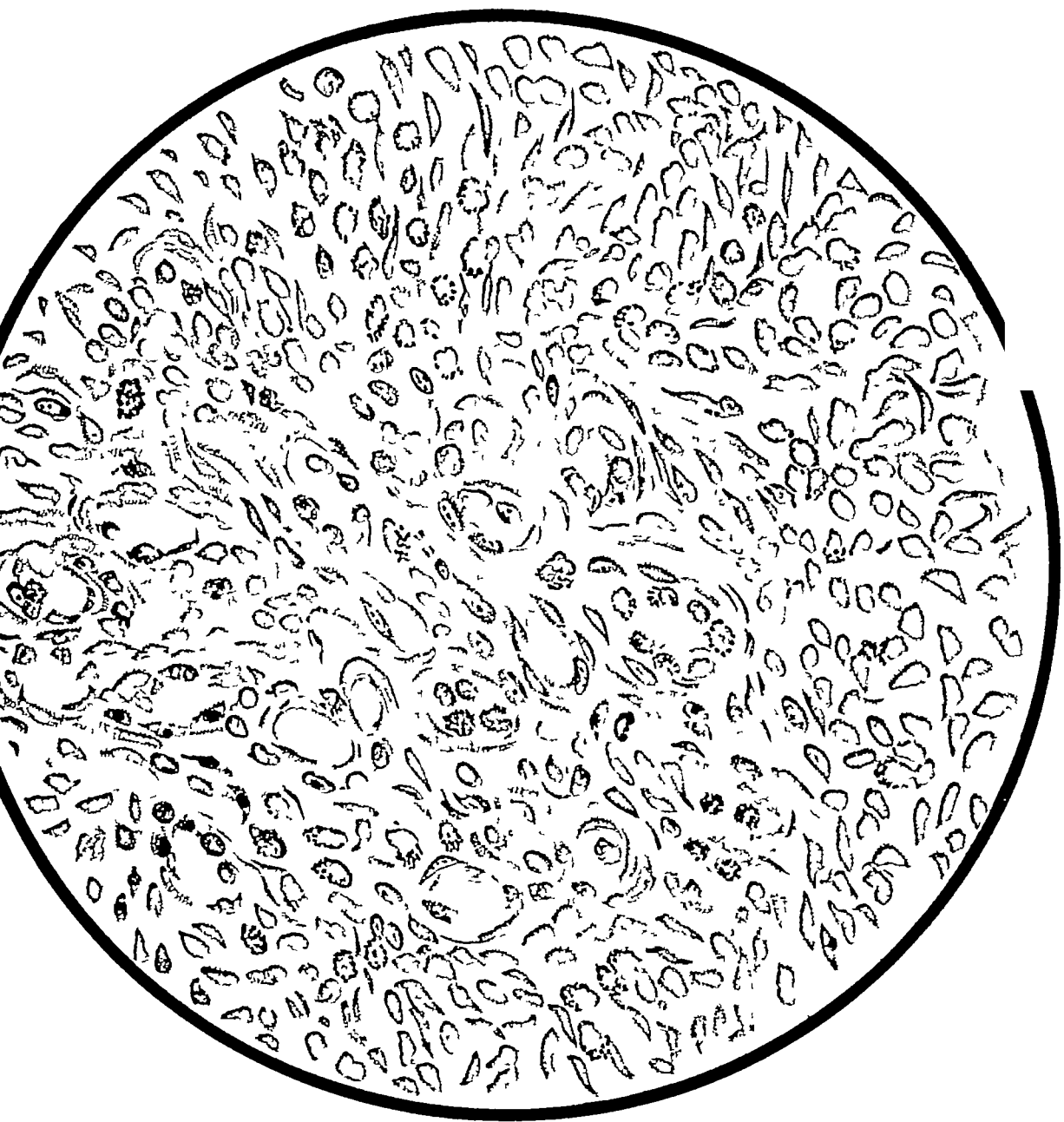
The significance of the tissue eosinophilia is hard to understand and it has very little to do with the blood eosinophilia that occurs in infections with intestinal parasites. The functions of the eosinophile cell itself are not clear. There are those who believe that these cells are effete polymorphonuclear leucocytes, the granules being the result of a breaking up of the cell and that the cell as a whole has little or no function. Others insist that the granules are secretory in function and secrete ferments. It is also pointed out that they take the place of the polymorphonuclear cells and are phagocytic to bacteria in exceptional conditions. It seems that this tissue eosinophilia is quite independent. The presence of eosinophile cells in the normal pituitary gland suggests that they may occur apart from infection.

An examination of a series of appendices removed surgically as well as from the post-mortem room has shown the presence of these cells in large numbers in the mucous membrane. They are well defined eosinophile cells with two or more lobes in the nuclei and coarse eosinophile granules occurring in numbers in

by the sixth day about half the eggs have disappeared. Since it is now recognized that the importance of hookworm infection to a community depends on the number of worms harboured by individuals rather than on the number of persons infected, and since the intensity of an infection is estimated by the number of eggs per gramme of faeces, it is clear that it is of the utmost importance that there should be no loss of eggs in the faeces to be examined, if reliable results are to be collected.

Lane (1923-24) still advocates centralization of hookworm survey work, and he draws attention to the good results he has obtained with his (direct centrifugal flotation) method, by using faeces that had been kept in water or in thymolized water at a temperature not above  $28^{\circ}$ — $30^{\circ}\text{C}$ . He also shows that higher temperatures have a bad effect on the results obtained by DCF, and so he recommends the use of refrigerating boxes, to be filled with ice at the central laboratory, despatched to the field, filled with faecal samples in thymolized water and returned. Though the method advocated would no doubt be practicable, it is beset with difficulties, and in a large country like India, where delay in transit of boxes would at times inevitably occur, the scheme would be liable to break-down, from melting of the ice. A further objection is, that efficient refrigerating boxes would be expensive, as they would have to be so well insulated, that they would remain at a low temperature for well over a week at a time, if they were to be used in India. Lane's work on this subject was done in England, presumably on faeces from people eating a normal European diet, and hence not nearly so liable to rapid fermentation, as are the bulky stools of vegetarian peoples. The writer put up nine different specimens of stools from Indians, both in water and thymolized water, after the directions of Lane, and he kept them at a temperature of  $25^{\circ}\text{C}$  for five days. The result was checked, not by Lane's DCF, but after Stoll's counting technique, and it was found that the counts on the day the stools were passed compared with the counts of stools preserved for five days showed a loss of eggs as follows: 32 per cent in stools in water, 36 per cent in stools in thymolized water, and 38 per cent in stools kept in tins. It is therefore clear that for Indian stools, preservation in water at a temperature of  $25^{\circ}\text{C}$  has little effect in preventing loss of eggs. Lane further states that there was no development of eggs in stools kept in water, in which they sank to the bottom of the tube, this was not the case in the writer's experience, for fermentation rapidly occurred even at the relatively low temperature of  $25^{\circ}\text{C}$ , and the gas formed caused the bulk of the faeces to rise to the surface of the liquid, where development of eggs proceeded. This was evidenced by finding free larvæ on four occasions while doing counts, and numerous eggs containing embryos were seen on all occasions. Therefore, although preservation of faeces in water or thymolized water at a temperature not above  $30^{\circ}\text{C}$  may be reliable for European stools, it is of little value for the stools of people whose diet is wholly vegetarian.

On account of the above objections to Lane's recommended method of faecal preservation for hookworm eggs, when applied to conditions in India, the writer has experimented with a number of fluids, in an attempt to find one that will



Eosinophile infiltration in the Submucosa of a chronically inflamed appendix.—Camera Lucida Drawing

*Comparison between fresh and preserved stools*

	Number of counts	Total eggs per gramme of fresh stools	Total e p g of stools preserved 1 to 18 days	Average e p g in fresh stools	Average e p g in preserved stools
Room temperature	41	170,600	170,700	4,161	4,163
Incubator 39°—42° C	28	98,000	100,400	3,500	3,585
TOTAL	69	268,600	271,100	3,893	3,927

These counts are each the average of two counts made by two observers, therefore the actual number of counts made is twice the number shown in the table

The counts made on fresh stools and on the same stools after preservation are remarkably close, and that fact that those made on preserved stools are slightly higher clearly indicates that there is no loss of eggs in this method of preservation. The reason that preserved stools give a slightly higher count than fresh faeces is probably not altogether a matter of chance, for during the progress of the work one has been impressed by the fact that counting preparations of preserved stools are remarkably clear, for the faecal matter is broken up into the finest particles by the prolonged soaking, and 'camouflage' is practically eliminated.

In the case of stools kept for fourteen to eighteen days, eighteen samples were kept at room temperature, and seventeen samples in the incubator. The counts of these have been included in the above table, but they are shown separately below to indicate that there is no loss of eggs in these longer-preserved specimens, which might have been masked by higher counts on those samples kept for shorter periods.

*Counts on stools preserved 14 to 18 days*

	Number of counts	Total eggs per gramme of fresh stools	Total e p g of preserved stools	Average e p g of fresh stools	Average e p g of preserved stools
Room temperature	18	72,300	72,500	4,017	4,025
Incubator 39°—42° C	17	67,800	67,700	3,988	3,982
TOTAL	35	140,100	140,200	4,003	4,006

Now, whatever may be the origin and functions of the eosinophile cells, it is certain that these cells emigrate to the deeper tissues as a result of some form of chemotaxis and their frequent presence in places where cell destruction or cell growth is abnormal, suggests that the presence of these cells in the submucous coat is the result of some chronic irritative or inflammatory process that existed or exists in the deeper layers

#### SUMMARY

(1) Eosinophilia of the mucous membrane of the appendix is a normal phenomenon at least in the tropics

(2) Eosinophile infiltration of the submucosa of the appendix is evidence of chronic appendicitis

In concluding I must express my indebtedness to Captain P N Basu, *IMS*, Professor of Pathology, for permission to carry on this work

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been found to operate successfully. The writer has recently employed it in a series of about eight hundred stools collected in the Dooars district of northern Bengal, and sent to the School of Tropical Medicine in Calcutta by rail.

In the first place, the faecal samples in the above experimental investigation were placed in ordinary cylindrical specimen tubes, these tubes cannot be corked tightly, and in all probability would leak if used for despatch of samples by rail. The writer consequently obtained small bottles of about 27 ccs capacity these are capable of being tightly corked. The dimensions of the bottles are as follows: height including neck 55 mm, diameter 35 mm, and internal diameter of neck 18 mm. Their price in Calcutta is only Rs 7 per gross (under one penny each) including corks. The first difficulty that arose in using the bottles was, that it was not possible to measure accurately enough the correct quantity of faeces by displacement between marks on the outside of the bottle, because the relatively large diameter of the bottles brought the two marks, between which faeces displacement was to be made, so close together, that a slight error in reading the level of the contained fluid would mean a considerable error in the amount of faeces introduced. The thickness of the glass added to this difficulty, and a further source of error was, that it was difficult to introduce portions of faeces into the bottles without touching the sides of the relatively narrow neck. To overcome these defects the writer made small measures of paper, which can be quickly and accurately made in the following manner.

The kind of paper found most satisfactory was fairly stiff writing paper. This is first cut into strips about 10 to 12 mm wider than the depth of the completed measure is to be, it is then cut into lengths about 2 mm longer than the circumference of the measure, rolled tightly round the piston of a hypodermic syringe of suitable size, the overlapping portion gummed, and the paper cylinder thus completed is slipped off the piston. The piston is marked with a ring, the exact distance from its distal end to give the number of cubic centimetres, which it is desired the measure shall contain. The position of this ring is easily obtained by utilizing the graduation on the barrel of the syringe. The paper is now pushed on to the piston as far as the marked ring, and the projecting paper is folded in against the end of the piston to form the bottom of the measure, and held by a dab of gum. The paper measure thus produced is an accurate measure of the desired capacity. It is rare to find a syringe piston with a flat end and sharp angles between the end and sides, but this difficulty may be easily overcome by having a small cylinder of hard wood made, the same diameter as the piston and with square cut ends, such a piece of wood makes a very satisfactory gauge. It was found that a 10 c c syringe produced a measure of a handy size, for a 4 c c measure made on its piston was about 21 mm in depth, and had a diameter a little over 15 mm.

For transportation of specimens, boxes were made which each contained fifty bottles in two layers of twenty-five each. A false bottom was fixed in the box about one inch above the true bottom, and in it twenty-five circular holes were cut of the correct diameter to hold the bottles. A double tray consisting of

The object of our investigation is first to establish definitely that cases of ascites which are clinically obscure and which are not of cardiac, renal or hepatic origin, are common in certain parts of India and then to throw as much light as possible on their ætiological factors

We decided to take up all cases of ascites admitted to the hospital and thus from 1st August, 1927 to 29th February, 1928, we have been able to observe 35 cases. Those cases, which from clinical examination showed obvious evidence of cardiac, renal and hepatic origin, were eliminated. Three cases showed myocardial and valvular lesions with heart failure. Eleven cases of hydræmic nephritis showed definite kidney lesions with albumen and casts in the urine, marked chloride retention and general anasarca. Two cases showed evidence of hepatic origin with irregular enlargement of liver, dilated veins and caput medusæ. Out of the 35 cases, then, 16 had to be excluded for the above reasons. The remaining 19 cases were submitted to systematic investigation.

### ÆTIOLOGY

*Geographical distribution in Behar and Orissa*—In a province with an area of 83,000 square miles, divided into 21 districts and with a population of 33 millions, observation restricted to admissions to one general hospital does not give a clear idea of the numerical incidence of the disease in relation to the total population. Nevertheless, a large number of admissions from the area where the hospital is situated gives some idea of its relative frequency in that area. Thus, we had 9 cases from Patna district, 4 from Muzaffarpur, 3 from Chapra and 1 each from Monghyr, Champaran and Gaya.

*Age*—Only adults seem to be affected, the disease being most common between 30 and 50 years, two of the cases were above 50.

*Sex*—All cases in our series were males. The fact that women in this province come less frequently to the hospital may explain their apparent freedom from the disease.

*Race*—No Europeans and Anglo-Indians were included in our cases.

*Religion*—The cases were equally distributed amongst Hindus and Mohammedans.

*Occupation*—The majority of our cases were cultivators.

*History of previous illnesses*—Fifteen cases gave a history of dysentery or diarrhœa. In some, it just preceded the collection of fluid in the abdomen and in others, it dated as far back as 2 or 3 years.

Twelve cases had a previous attack of malaria or kala-azar, 12 gave a history of typhoid, 1 of syphilis, and 2 of alcoholic indulgence.

*Mode of onset*—The onset is insidious. Gastro-intestinal disturbance is a frequent accompaniment. Flatulence, acid eructations, loss of appetite, diarrhœa and, in some cases, constipation are the common complaints. Debility is always present. The swelling of the abdomen is gradual and progressive, it disappears at times to reappear within a short period. In 3 to 5 months, the collection of fluid becomes excessive and the patient comes to the hospital.

The tube is now submitted to D C F in the usual way, and if a positive result is obtained, the mixture in the bottle is poured into a flask, the correct amount of N/10 sodium hydroxide to give the proper dilution for counting by Stoll's method, when added to the 18 c cs already in the flask, is measured in a graduated cylinder. This is poured into the bottle in successive portions, and the latter is thus thoroughly rinsed out, the rinsings of course being added to the flask, which now contains a proper dilution for counting.

The size of the boxes and the number of bottles they contain is purely arbitrary, and, in some cases, it would probably be found better to have boxes containing one hundred or even more bottles, but the most convenient size of boxes for any given conditions would have to be determined. It would depend upon the length of time they took to reach the laboratory, and the number of microscopists at work, and it should be arranged so that collection of specimens in the field and their examination in the laboratory proceeded at about the same rate. In this way all the persons concerned would be working full time, and there would always be a supply of bottles of antiformin in the field, and bottles of faecal samples to the laboratory. It is especially important that a supply of bottles should be always available for the collectors, because it is absolutely necessary for the success of the method that faeces are not kept in tins from one day to the next, pending the arrival of bottles to contain them, for the loss of eggs even in one day is considerable.

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TABLE I  
*Renal efficiency tests*

Case No.	Chloride excretion Percentage.	UREA CONCENTRATION TEST PERCENTAGE OF URINARY UREA			Efficiency
		Before urea	1 hour after	2 hours after	
I	0.68	3.0			Good
II	0.5	3.2			Good
III	0.38	1.0	1.5	1.6	Bad
IV	0.66	3.5	3.8	4.2	Good
V	0.6	0.9	1.6	2.3	Fair
VI	0.56	2.4	4.2	4.4	Good
VII	0.88	1.0	2.6	4.0	Good
VIII	1.6	1.4	2.5	3.2	Good
IX	1.4	0.6	1.7	2.1	Fair
X	0.58	2.0	2.8	3.0	Good.
XI	0.50	2.6	3.8	4.2	Good.
XII	0.98	0.6	2.2	2.5	Good
XIII	0.8	0.4	1.3	3.0	Good
XIV	0.26	0.15	0.45	1.8	Bad
XV	0.37	0.8	1.9	2.0	Fair
XVI	0.56	1.6	1.9	2.1	Fair
XVII	0.45	0.6	1.2	1.9	Bad
XVIII	0.8	2.3	3.2	3.2	Good
XIX	0.56	0.8	1.4	1.8	Bad

*Cardiac efficiency tests*

The pulse rate after complete and prolonged rest, in 5 cases was 90 to 100 beats, in 6 cases, 80 to 90 and in 5 cases, below 80 per minute. By the exercise tolerance test, only 5 cases out of 16 showed an appreciable diminution in the reserve force of the heart. In majority of the cases, the systolic pressure varied from 100 to 115 mm and the diastolic pressure from 60 to 90 mm of Hg. An increased pulse rate at rest points to some infective focus. The diminution in the reserve force in 5 of the cases was probably due to the mechanical effect of fluid in the abdomen.



TABLE II

## Hepatic efficiency tests

Case No	Liver	BLOOD-SUGAR IN MG PER 100 CCS			Rise of blood-sugar in mg per 100 ccs	Efficiency	HEMOCLASIC CRISIS W B C PER C MM			Rise or fall of W B C count.	Efficiency
		Before lavulose	45 minutes after	90 minutes after			Before milk	20 minutes after	40 minutes after		
I	+	95	130	132	37	C					
II	+	83	80	129	46	C					
III	+	83	126	150	67	C	5,600	6,250	5,900	+650	B
IV	+	80	112	129	49	C	4,688	5,624	6,248	+1,560	B
V	+	95	130	132	37	C	5,936	6,250	7,186	+1,258	B
VI	—	140	180	180	40	C	5,900	6,200	7,500	+1,600	B
VII	+	115	133	162	47	C	7,500	7,812	8,750	+1,230	B
VIII	—	70	80	70	10	A					
IX	+	70	75	80	10	A	13,400	12,000	15,936	+2,496	A
X	—	81	106	118	37	C	3,744	4,056	4,680	+936	B
XI	—	120	140	130	20	B	5,312	7,250	7,562	+2,250	A
XII	—	102	112	112	10	A	3,752	3,596	5,000	+1,258	B
XIII	—	100	120	118	20	B	4,687	6,162	7,188	+2,500	A
XIV	—	118	118	137	19	B					
XV	—	90	134	130	44	C	5,936	6,562	6,562	+636	B
XVI	—	108	131	132	24	B	6,250	7,812	8,124	+1,864	B
XVII	—	87	125	156	69	C	5,000	6,250	6,625	+1,625	B
XVIII	—	110	140	160	50	C	6,250	6,250	8,268	+1,918	B
XIX	+	106	106	125	19	B	5,937	7,812	8,437	+2,500	A

— indicates liver not enlarged

+ indicates liver enlarged varying from being just palpable to over 2 finger's breadth

Rise of blood-sugar up to 10 mg liver efficiency = good (A)

" " " from 10-30 " liver efficiency = slightly deranged (B)

" " " over 30 " liver efficiency = definitely deranged (C)

Leucocytic response over 2,000 shows no appreciable derangement (A)

The general appearance of the male agrees with the usual descriptions, so will not be referred to further, but as the writer has been able to make out certain characters in the tail, which have not been hitherto described, it is proposed to discuss the anatomy of this portion in detail. In the natural state the tail is wound in about three spiral turns, which makes minute examination of this part difficult, as the worms cannot be rolled under a coverslip with ease. The cloaca is situated about 0.11 mm in front of the tip of the tail. There are two pairs of minute sessile papillæ near the tip of the tail, and in uncleared specimens nine pairs of pedunculated papillæ can be seen in relation with the cloaca. Five pairs of these are pre-anal, and four pairs are post-anal, they are about 7  $\mu$  to 8  $\mu$  in length and end in a small drum-stick-like knob, and they are of the type one sees typically associated with caudal alæ. In the present instance they are seen to support a distinct narrow cuticular flange, but this can only be seen if one gets the worm in a certain position, with the flange projecting into the curve of the tail when the worm is lying on its side. In cleared specimens the papillæ cannot be made out with certainty, nor can the caudal alæ be seen, but when in this condition, a semi-circular flap or papilla of cuticle can be clearly made out, it is about 8  $\mu$  from base to tip, and is of about the same width. In uncleared worms these adanal flaps are seen to lie mesial to the alæ, to which they have no attachment. There are distinct cuticular striations about 3  $\mu$  apart on the ventral surface of the tail, they begin just in front of the cloaca, and end about opposite the proximal termination of the long spicule.

The spicules are unequal in length and dissimilar, and they are essentially the same as previous workers have described. The length of the two spicules is about 0.5 to 0.52 mm and 0.2 to 0.21 mm. The long spicule is composed of a stout proximal tubular portion about the same length as the short spicule, and it ends in a long thin lash. Where the tubular part narrows into the lash an irregular oval opening is seen, similar to the opening of a bamboo, which has been cut on a slant. The short spicule is stout for its whole length and is in the form of a grooved channel, it becomes gradually thinner from before backwards, and it appears to end in a bluntly rounded point. But the shape of its tip could not be determined with complete certainty for it was overlying the gubernaculum in both specimens. The stout portion of the long spicule and the whole of the short spicule, exhibit transverse markings with fine granular stippling. The gubernaculum, when seen from the side, appears to be merely a stout block of chitin crescentic in shape, but when viewed from before backwards in a vertical position, optical section shows that it is in the form of a U with the open end facing ventrally. The arms of the U are of unequal length, the long one being about 40  $\mu$  from base to tip, and the short one about 28  $\mu$ .

In the above account there are several differences from existing descriptions by other workers, which raise a doubt in one's mind whether different species have not been described under the name *Filaria bancrofti*. But the outstanding fact is, that with the exception of the tail of the male, the anatomy of the worms is similar in all cases, therefore it is probable that the variations given by different

Three of these 5 cases were positive to both the formolgel and urea-stibamine tests, whilst a further two cases gave a positive formolgel and urea-stibamine tests without any enlargement of the spleen. We regard positive formolgel and urea-stibamine reactions as diagnostic of kala-azar(10). Thus 31 per cent of our series were associated with kala-azar. Only 1 case had strongly positive Wassermann reaction. The calcium content of the serum was estimated by Krammer and Tisdall's method(11) in 15 cases. No appreciable variation was observed.

*The presence of specific agglutinins*

Table IV gives agglutination reactions of the sera to various organisms. Seven cases out of 19 showed the presence of agglutinins to *B. typhosus*,

TABLE IV

*Showing the results of culture of the stools and of agglutination tests with sera taken from ascites cases against different organisms*

Case No	<i>B. typhosus</i>	<i>B. paratyphosus</i> A	<i>B. paratyphosus</i> B	<i>B. dysenteriae</i> , Shiga	<i>B. dysenteriae</i> , Flexner	Stool culture.
I	-	-	-	-	-	<i>B. Faecalis alkaligenes</i> isolated
II	-	-	-	-	-	-
III	-	-	-	+ $\frac{1}{20}$	+ $\frac{1}{20}$	-
IV	-	-	-	-	+ $\frac{1}{20}$	-
V	-	-	-	-	+ $\frac{1}{20}$	<i>B. flexner.</i>
VI	-	+ $\frac{1}{10}$	+ $\frac{1}{10}$	+ $\frac{1}{20}$	+ $\frac{1}{20}$	-
VII	-	+ $\frac{1}{20}$	-	-	+ $\frac{1}{20}$	-
VIII	-	-	-	-	+ $\frac{1}{20}$	-
IX	+ $\frac{1}{20}$	-	-	-	+ $\frac{1}{20}$	-
X	+ $\frac{1}{20}$	-	-	+ $\frac{1}{20}$	+ $\frac{1}{20}$	-
XI	-	-	-	+ $\frac{1}{20}$	+ $\frac{1}{20}$	-
XII	+ $\frac{1}{20}$	+ $\frac{1}{20}$	-	-	+ $\frac{1}{20}$	-
XIII	-	-	-	-	+ $\frac{1}{20}$	-
XIV	-	+ $\frac{1}{20}$	-	-	+ $\frac{1}{20}$	-
XV	-	-	-	-	+ $\frac{1}{20}$	-
XVI	+ $\frac{1}{20}$	-	-	-	+ $\frac{1}{20}$	-
XVII	-	-	-	-	+ $\frac{1}{100}$	-
* XVIII	-	-	-	-	+ $\frac{1}{20}$	-
* XIX	-	-	-	-	-	-

+ indicates complete agglutination in the dilution of the serum noted



pair of broad adanal papillæ. There was a suggestion of other papillæ in the region of the cloaca, but these were extremely shadowy so that neither their number nor even their actual existence could be definitely decided upon. But when examined in alcohol and rolled to just the correct angle, one specimen exhibited narrow caudal alæ each supported by nine pedunculated papillæ, which were so distinct that there was no room for doubt as to their number nor as to the nature of the structure to which they were attached. In the second specimen, which had the tail more sharply bent, and could not be rolled readily in consequence, all that could be seen were three indefinite pre-anal and two post-anal papillæ, these did not appear pedunculated and the caudal alæ could not be made out.

Taking Leiper's opinion, that the papillæ on the tail of the male are subject to slight individual variation in number as correct, it seems reasonable to accept the view of the majority of workers who have described this worm, and to consider that there may be nine to twelve pairs of papillæ in relation with the cloaca. The small sessile papillæ near the tip of the tail are not of the same type, and it would probably be better to enumerate them separately. The writer as the result of his observations thinks that the fact that the papillæ in relation with the cloaca are pedunculated, and that they support narrow caudal alæ, should be added to existing descriptions, and also that the pre-anal portion of the ventral surface of the male is marked by definite transverse striations, should be included.

Although it was not possible to obtain a ventral view of a male tail and thus make a drawing of the view in this position, a diagram of what is almost certainly the appearance of the worm has been included in the figures.

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#### EXPLANATION OF PLATE LIV

- Fig 1 Anterior end of female, lateral view  
 „ 2 Tail of male, lateral view  
 „ 3 Enlargement of male cloaca to show adanal papillæ  
 „ 4 Tail of male to show gubernaculum in antero-posterior view  
 „ 5 Tail of male, lateral view of uncleared specimen showing papillæ and caudal alæ  
 „ 6 Diagram of tail of male, ventral view.

indicating that it was a transudate. In most cases this was further confirmed by Runeberg's test(12). In one case only, the ascitic fluid, with a specific gravity of 1019, was an exudate and when injected into a guinea-pig produced severe peritonitis with death of the animal in 24 hours. Nothing of importance was noticed at the post-mortem examination. The total cell count on the average was above 100 per cmm consisting mostly of endothelial cells, a few lymphocytes were always present, but there was remarkable absence of any polymorphonuclear cells except in the case in which the fluid was an exudate. None of the fluids produced any growth on cultural examination, either aerobic and anaerobic. Young guinea-pigs were inoculated intraperitoneally with the ascitic fluid and, when examined after about 5 to 6 weeks' observation, showed no signs of any disease. In several instances, more than one injection had been given. The study of the fluid after the second and third tapping did not reveal any altered cell count. The fluid can, therefore, be regarded as non-tuberculous, sterile and mechanical in origin.

### DISCUSSION

The results of our investigation of nineteen cases definitely prove the existence of a group of ascites cases which are non-cardiac and non-renal in origin.

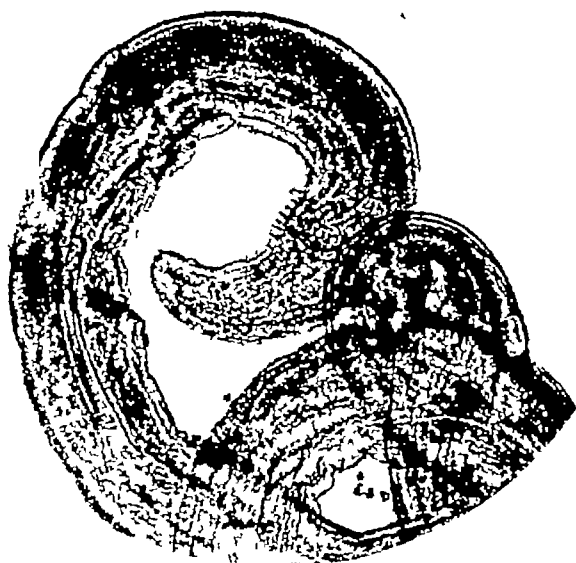
Five cases out of sixteen, or 31 per cent, show association with kala-azar. These five, e.g., Cases IV, VI, VIII, XVI and XVII also had a previous dysenteric infection. It is difficult to ascertain to what extent kala-azar is responsible for the causation of ascites. Rogers has described a fine fibrosis of the liver and the development of ascites in long-standing kala-azar cases. In the kala-azar ward of the Patna General Hospital, remarkable improvement has been noticed in 2 cases with ascites with urea-stibamine treatment. Unfortunately, it was not possible to apply the therapeutic test to these cases showing a positive kala-azar reaction. But the therapeutic test on the improvement or otherwise of a morbid condition can hardly be relied upon as establishing an aetiological relationship. Three of these cases also showed a definitely deranged hepatic function. Presuming for the moment that the morbid changes in these cases are the result of kala-azar, and the positive agglutination to dysentery organisms is a superimposed condition, we will exclude these cases, as of kala-azar origin, and concentrate our attention on the remaining 'non-kala-azar' cases.

Out of the fourteen 'non-kala-azar' cases, eleven gave evidence, and three gave no evidence, of a previous dysenteric infection. Eight cases showed a definitely deranged, four a slightly deranged and two a normal hepatic function.

The ascitic fluid examined in eleven cases proved to be a transudate in 8, an exudate in 1 and of doubtful origin in 2 cases. No morbid changes were noticed in any of the guinea-pigs injected with the fluid except in the case of the exudate.

The association of the disease with a previous dysenteric infection in the majority of cases, as ascertained by the clinical history as well as by the agglutination reaction, is clearly established. This relationship, however, is only confirmatory and has been noted by previous observers in India. The possibility of a

PLATE LV



Microphotograph of tail of male, uncleared. The most posterior of the nine papillæ cannot be seen as it is out of focus, and the caudal alæ only appear as a faint light area, owing to their delicacy.

by the fact that we were unable to demonstrate tubercle bacilli in the fluid or to palpate after tapping any enlarged glands or a thickened omentum in any of the cases, further, none of the guinea-pigs showed signs of tuberculosis after injection of the fluid, although in several instances more than one injection had been given

A simple chronic peritonitis, diffuse and progressive in character, associated with ascitis, due to infection by micro-organisms of a lower grade of virulence, has been described. This condition is sometimes called chronic proliferative or chronic indurative peritonitis. Similarly, the cases under review have been described as chronic dysenteric peritonitis or chronic superior peritonitis. In these cases, however, the evidence of any local inflammatory reaction or infection at the present stage of the morbid process is lacking. The existence of an inflammatory or irritative process at an earlier stage of the illness which has left a thickened peritoneum, cannot, however, be denied. Further, it is generally held that the ascites is usually caused by the chronic peritonitis in such cases, but it is conceivable that in certain circumstances the reverse might hold good. Thus, a long-standing collection of fluid in the peritoneal cavity, caused by interference with the normal absorptive process, might produce proliferation of the endothelial cells thus causing a thickened peritoneum.

The appearance of the fluid after repeated tapings confirms the view that the absorption is defective. In health, secretion of fluid, mainly by the great omentum, into the peritoneal cavity and its absorption therefrom is so evenly balanced that there is just enough fluid to keep the serous surfaces moist and free from friction. In morbid conditions, this state of equilibrium is disturbed and the amount of fluid may become excessive with consequent ascites. The peritoneum covering the under surface of the diaphragm differs from the rest of the peritoneum in its greater power of absorption. Buxton(13) has shown how rapidly absorption takes place through the lymphatics of the diaphragm. Any interference with the vitality of the endothelial cells lining this part of the peritoneum and blockage of the adjoining lymphatics can, therefore, prevent normal absorption. The dysenteric toxin arising from the infected bowel may possibly be responsible for this interference. The same agent, absorbed into the portal circulation, may also cause damage to the hepatic cells. We consider it probable that both these agencies may be at work in the production of these cases and that only those cases develop ascites which are affected with extensive ulceration reaching the transverse colon which, in its turn, causes damage to the adjoining endothelial cells and the lymphatics with a consequent thickening of the peritoneum in that region.

#### SUMMARY AND CONCLUSIONS

1 Cases of ascites of non-renal, non-cardiac and apparently non-hepatic origin are common among adult Indian males in Behar. The poorer classes are mostly affected.

2 It is a chronic and progressive disease associated with bacillary dysentery (84 per cent) and with kala-azar (31 per cent).

The present series of investigations was undertaken with a view to determining whether any change directly attributable to leprosy could be detected in certain of the physical properties of the blood and serum and to correlate these changes if possible with the type and stage of the disease and the sedimentation rate of the erythrocytes

(1) *Specific gravity of whole blood*

For the determination of this property, the simple and relatively accurate method described by Rogers (1913) was employed. This consists in making up a series of mixtures of glycerine and distilled water so as to give a range of specific gravity from 1046 to 1064. Blood was drawn from the median basilic vein into a perfectly dry and sterile all-glass syringe and one drop quickly added to each of a series of test-tubes containing about 5 ccs. of the glycerine-water mixtures of different specific gravity. The specific gravity of the blood is represented by the particular glycerine-water mixture in which the drop of blood prior to disintegration remains momentarily suspended in the body of the fluid. Simultaneous estimations were made and the final result was taken as the mean of the independent readings.

Thirty cases representing different types and stages of the disease were investigated and the following tables give a critical analysis of the results obtained —

TABLE I

*Comparison of the Specific gravity of the Blood with the Type and Stage of the disease, according to Muir's classification*

'A' type comprising 'A <sub>1</sub> ' and 'A <sub>2</sub> ' cases	Specific gravity of blood.
G	1055
A. G	1054
N H	1053
A S	1054
D M	1053
A R.	1055
A H	1057
M P	1056
S R.	1050

Group average—1054

# A SIMPLE METHOD OF PRESERVING FÆCES CONTAINING HOOKWORM EGGS

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As long ago as 1919, Lane drew attention to the obvious advantages that would accrue, if fæces could be transported to a central laboratory for examination for hookworm eggs. The usual method in vogue in the conduct of a hookworm survey, is for a fairly large staff of trained microscopists to travel slowly through a district, setting up temporary laboratories in suitable places. This entails the expenditure of considerable sums of money for travelling and housing, and it is often difficult to find a suitable building for a temporary laboratory. If, however, fæces could be collected and placed in a preservative, which would prevent loss of hookworm eggs, all that would be needed in the field would be a small untrained staff of collectors under the supervision of a reliable man, not necessarily skilled in laboratory technique. Such a staff would be much cheaper to maintain, and more reliable results could be anticipated from the examination of material in a permanent well-equipped laboratory, than in temporary and frequently inconvenient field laboratories, where makeshifts have often to be resorted to.

Although Lane (1919) showed that certain preservatives kept hookworm eggs in a good state for detection by his levitation method, his plea for centralization of this work has received little attention, and the almost universal custom in conducting hookworm surveys is still to collect fæces in tins, and to examine them in the field as soon as possible after collection, but even so, one or more days often elapses between collection and examination of material. A general impression seems to have arisen, that it does not matter to keep stools in tins for one or two days, even without ice, because normal looking eggs are still present. In a recent paper, however, Maplestone (1928) has shown, that loss of hookworm eggs from fæces kept in tins at ordinary temperatures is considerable in the first twenty-four hours, and that it proceeds with a fairly constant regularity, so that

Table III shows the relationship between the sedimentation of erythrocytes and the specific gravity of the blood

TABLE III

Sed rate (0—20)	Sp gr of blood	Sed rate (21—40)	Sp gr of blood	Sed rate (41—60)	Sp gr of blood	Sed rate (over 60)	Sp gr of blood
5·7	1062	21·0	1055	42·0	1052	62·1	1053
6·5	1055	21·0	1056	45·0	1053	66·2	1049
10·5	1057	23·7	1056	47·0	1057	70·1	1054
12·2	1053	29·0	1054	49·0	1050	72·6	1052
14·5	1054	30·5	1084	50·0	1050	76·0	1047
16·4	1053	32·7	1054	50·5	1053	79·2	1047
18·5	1054	35·0	1056	50·5	1054	.	.
		.		53·5	1051		.
		.		54·2	1050		.
.		..	..	55·7	1048	..	.
	Average 1055		Average 1054		Average 1052		Average 1050

There is, therefore, some correlation between the sedimentation rate of the erythrocytes and the sp gr of the blood in that an increased sedimentation rate tends to be associated with a relatively low sp gr

## (2) *Fragility of the Red Blood Corpuscles*

This was estimated in the usual way by making up a series of solutions of NaCl in distilled water so as to give a range of concentrations of NaCl between 0·50 per cent and 0·30 per cent, with a mean difference of 0·02 per cent between any two adjacent solutions in the series. About 6 drops of blood obtained by finger-prick were received in a paraffin watch glass and of this 10 cmm were quickly transferred to each of a series of tubes containing the range of NaCl concentrations. Each tube was quickly inverted several times, the contents thoroughly mixed and the tubes set aside to stand for 30 minutes at room temperature. At the end of this period each tube was spun in the centrifuge for 5 minutes and the readings taken.

Twenty-eight cases representative of all types and stages of the disease were tested in this way. Without going into unnecessary detail it may be stated that we could find no very great variations from normal in the majority of cases. Haemolysis began on the average at a concentration of NaCl of 0·43 per cent which is 0·01 per cent lower than the figure usually given for adult Europeans. Lysis of the corpuscles was complete between 0·34 per cent to 0·30 per cent.

preserve hookworm eggs in fæces for a sufficiently long period, and without recourse to réfrigerating boxes Antiformin of 2 per cent strength in water has proved effective under these conditions This preservative does not keep all the eggs unaltered, for many showed marked degeneration, especially in the specimens kept from fourteen to eighteen days, but they are easily recognized even when extensively degenerated, because counting preparations of these long-soaked stools are remarkably free from 'camouflage' Fermentation also occurs, with rising of fæces to the top of the fluid, but the eggs undergo no development They are not killed, however, by twenty-four hours immersion in 2 per cent antiformin, for cultures made from fæces treated in this way produced as many larvæ as fresh stools During the time the experiment about to be detailed was being carried out, the room temperature never fell below  $28^{\circ}\text{C}$ , and most of the time it was about  $33^{\circ}\text{C}$ , further, as a more severe test of the method than it is ever likely to undergo under natural conditions, a certain number of tubes were placed in an incubator ranging between  $39^{\circ}\text{C}$  and  $42^{\circ}\text{C}$  the whole time

*Experiment*—Two separate portions of each stool to be tested were measured by displacement of 3 ccs of N/10 sodium hydroxide on the day they were passed, and counted after Stoll's method A count of each of these two dilutions was made by the same two observers, and the average of the four counts was taken as the standard of that stool Ordinary cylindrical specimen tubes of about 30 ccs capacity were marked with a diamond at 20 ccs and 23 ccs from the bottom, they were filled to the 20 ccs mark with 2 per cent antiformin, and fæces were gradually added until the fluid rose to the 23 ccs mark These tubes were corked and kept for varying periods, some were kept in a cupboard, which admitted light through its glass doors, and others were placed in an incubator, which never fell below  $39^{\circ}\text{C}$ , and for most of the time was  $42^{\circ}\text{C}$  At first a tube from the cupboard and a tube from the incubator were counted after keeping for only a few days, but when it was found, that there was no loss of eggs, the time was gradually extended until up to eighteen days the number of eggs remained unaltered Longer periods than this have not yet been tried, for in most instances eighteen days is more than sufficient

Stools from twelve different individuals were examined, and they were typical stools of Indians, they were taken without other selection than that they all had fairly high egg counts This was done, so that slight loss of eggs might be detected with a fair degree of certainty, a thing which would be difficult to determine if only one or two eggs per counting preparation were normally present As the different counts on any one stool necessarily varied, being sometimes below and sometimes above the standard count for that stool, owing to the unequal distribution of eggs, the standard counts for all the stools have been added together, each having first been multiplied by the number of counts made on the same stool at later dates This total has been compared with the sum of all the counts made on the stools after preservation for whatever length of time they were kept, and thus any variation due to the unavoidable error of the method has been minimized, as far as possible



fell between the heavy marks on the stalagmometer tube. The whole procedure was watched through the inner glass door of the incubator thus avoiding fall of temperature during the experiment. In all, 22 sera from different types of leprosy were examined and in both the reacting and non-reacting phases of the disease.

A preliminary series of experiments under identical conditions was run using distilled water in place of serum in order to find the drop-number of the instrument at the temperature of the experiment. An examination of the surface tension of the serum in 7 healthy controls was also carried out. An extended series of observations showed that the 'drop-number' of the particular instrument used was 22.2 at 37°C. the surface tension of distilled water at the same temperature may be taken as 70 dynes per cm. and its specific gravity as 0.994. The specific gravity of serum was taken as 1.028 (Hammersten and Hedin). Calculation is effected by using the formula  $\frac{\gamma^1}{\gamma^2} = \frac{n^2 \times d^1}{n^1 \times d^2}$  where  $\gamma^1$  and  $\gamma^2$  represent the surface tensions of water and of the unknown respectively,  $n^1$  and  $n^2$  the number of drops and  $d^1$  and  $d^2$  the specific gravities of the two liquids.

Our results may be tabulated as follows —

(1) Surface tension of normal sera (mean of 56 observations)—63.3 dynes per cm.

(2) Surface tension of leprous sera (mean of 90 observations)—62.4 dynes per cm.

(3) Surface tension of 'reacting' leprous sera only (mean of 42 observations)—62.4 dynes per cm.

Comparing the surface tension of the serum with the sedimentation rate of the erythrocytes one obtains the following results —

Sedimentation rate	Surface tension of serum (group average) in dynes/cm
0—20	63.2
21—40	62.9
41—60	62.1
Over 60	62.2

*Discussion*—We are fully conscious of the fact that the stalagmometer is not a reliable instrument for the estimation of the *absolute* values of the surface tension of liquids particularly those of a semi-colloid nature. Ferguson (1915) drew attention to the errors inherent in the method and this point has been more recently elaborated by the studies of du Noüy (1926). In addition to the possible sources of error indicated above, the factors of time, temperature and agitation of the surface layer all play a part in surface tension estimations. It would appear that even the tensiometer method (which may be regarded as the most reliable of those at present at our disposal) does not give accurate results when

In order to test whether this method of preservation would permit of diagnosis by Lane's D C F, and counting by Stoll's, method on a single sample, a certain number of tubes were put up which contained 20 ccs of antiformin and 4 ccs of fæces, measured by displacement of the liquid. After storing these samples for a given number of days, either at room temperature or in the incubator, the contents of the tubes were thoroughly mixed by shaking, 6 ccs of the mixture were drawn up with a coarse pipette and transferred to a D C F tube, the precaution being taken to keep the tube agitated while this was being done, so as to maintain an even suspension. The easiest way of measuring 6 ccs of the above mixture was found to be done by running 6 ccs of water into a centrifuge tube, and marking the point it reached with a diamond scratch on the glass, portions of the mixture were then added to the empty tube with the pipette, until the mark was reached. Positive results were obtained in all cases even after eighteen days in the incubator, but it was not determined whether the number of eggs obtained from the first spin of one of these preserved specimens was as high as that obtained from the first spin of a sample of the same stool on the day it was passed. On this account the reliability of D C F for stools preserved in this manner is not conclusive, because the number of eggs in the stools was rather high in the first place, and so there might be a considerable falling off in the number of eggs that came up by D C F and it would still be positive.

The remainder of the mixture suitably diluted with N/10 sodium hydroxide and counted, gave results closely approximating to counts made on specimens of the same stool when 3 ccs had been measured into 20 ccs of antiformin and kept for the same length of time. This indicates, that the method of removing one quarter of the mixture for D C F leaves 3 ccs of stool for counting, which for practical purposes is sufficiently accurately measured. Since this work was done, a more accurate test was made by placing 20 ccs of stool in a flask containing 100 ccs of 2 per cent antiformin, withdrawing 6 ccs of this mixture every day for examination by D C F, and counting the eggs obtained from the first spin each day. Allowing for days on which counts were not done, the experiment lasted for twenty-three days, and the following are the counts for successive days—30, 52, 43, 66, 46, 16, 16, 32, 44, 47, 38, 33, 27, 36, 26, 54, 32, 21. This, although a single example, indicates that D C F is reliable for preserved fæces for at least three weeks. A further confirmation of the value of this method of preservation of fæces is, that in a series of nearly eight hundred stools treated in this way, about 10 per cent of them were positive by D C F, and negative when counted by Stoll's method, and only one of the same series was positive by Stoll's method and negative to D C F. From the above facts it seems clear, that a solution of antiformin in water in a strength of 2 per cent is an efficient preservative for hookworm eggs in fæces, for more than a sufficiently long period to allow of fæces being sent to a central laboratory for examination.

The work detailed above was, however, carried out in the laboratory and for its practical application to field conditions, a few slight modifications had to be introduced. The following technique has accordingly been devised, and it has

- HENDERSON (J M) (1927) *Proceedings Far Eastern Assocn of Tropical Medicine*  
7th Congress
- ITURBE (P M) (1927) *Gac Med de Caracas*, Vol 34, No 1
- JOHLIN (J M) (1928) *Jour of General Physiology*, Vol XI, No 3
- KNAPP (H H G) (1915) *Ind Med Gaz*, Vol 50, No 3
- LABERNADIE (V) and ANDRE  
(Z) (1927) *Bull Soc Path Exot*, Vol 20, 839
- LAGANE (L) and COLOMBIER  
(P) (1913) *Bull Soc Path Exot*, Vol 6, No 6
- LANDFIRO (F) (1926) *Compt Rend Soc Biol*, Vol 95, No 34
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- LEMANN (I I) *et alia* (1927) *Amer Jour Trop Med*, Vol 7, No 1
- MARCHAND (H) (1922) *Bull Soc Path Exot*, Vol 15, No 3
- MITSUMA (K) (1923) IIIe Conference Internationale de la lepre, Strasbourg
- MUIR (E) (1928) *Ind Jour Med Res*, Vol XVI, No 1
- PALDROCK (A) (1925) *Derm Woch*, Vol 8, No 27
- PARAS (E M) (1926) *Philip Jour Sc*, Vol 30, No 2
- PARAS (E M) (1927) *Philip Jour Sc*, Vol 33, No 2
- PRINGAULT (E) (1912) *Arch de l'inst Past*, Tunis, No 4
- PUXEDDU (E) (1924) *Riforma Med*, Vol 40, No 22
- ROGERS (L) (1913) 'Cholera and its Treatment,' Oxford Medical Press
- SADI DE BUEN (1916) *Bol Inst Nac Higiene de Alfonso XIII*, Vol 12,  
No 48
- UNDERHILL (F P) *et alia* (1920) *Jour Exp Med*, Vol 32, No 1

two sheets of thin wood one inch apart was made, and twenty-five holes were cut in the upper sheet of this tray to hold the second lot of bottles. A narrow flange of wood was placed inside the box to take the tray, and this was arranged at such a height as to allow the bottom of the tray when in position, to come in light contact with the corks of the lower layer of bottles, and to hold them in position. The lid of the box was attached by hinges, and when closed it just touched the corks of the bottles in the tray, in this way the need for straw or other loose packing was done away with. The box was closed by a hasp and staple and a padlock, the field collector having one key and the other being left in the laboratory. The dimensions of a box to hold fifty bottles, such as are described above, is about  $12 \times 12 \times 6\frac{1}{2}$  ins. The recesses in the tray and false bottom were marked from one to fifty, and inside the lid of the box a sheet of paper was fixed with drawing pins, and this was ruled with suitable columns to contain the particulars desired, regarding the specimens in the box.

The procedure is to place 20 ccs of antiformin solution in each bottle at the laboratory, cork them tightly, place them in a box, and despatch it to the field collector. In the field it is found best to still utilize the usual small tin pill boxes with plain paper on the lids for recording particulars, for the actual collection of stools from individuals, as they cannot be trusted to fill one of the paper measures accurately. On the day the collector receives the tins of fæces, he fills a paper measure from each tin and drops one into each bottle, at the same time he enters the particulars on the sheet of paper in the lid of the box opposite the number corresponding to the number of the recess in which any given bottle lies. The paper measure very soon becomes un-gummed, allowing the fæces to escape and mingle with the fluid.

In filling a paper measure with anything but liquid fæces, it is best to transfer only a small portion of fæces at a time from the tin to the paper, because one has to be careful that the fæces completely fills the angle between the sides and the bottom of the measure, and this is not easily done if a large piece of stool is put into it at the beginning.

Although the corks were pressed as tightly as possible into the bottles, it was found that the pressure of gases from fermentation loosened quite a number of them so that leakage occurred, after this the corks were tied in with a loop of twine fixed round the neck of the bottle, and from this time onward, there was not a single case of leakage.

After unpacking at the laboratory, the first thing to do is to extract the paper from the bottles with a pair of forceps. The DCF centrifuge tubes have all been previously marked with a diamond at the point which 6 ccs of fluid reaches in them. A bottle of fæcal material is now vigorously shaken, and 6 ccs of the contents are transferred to a centrifuge tube by means of a coarse pipette, taking care to keep the bottle agitated the whole time this process is going on. As there was 4 ccs of fæces and 20 ccs of antiformin originally in the bottle, 1 cc of fæces and 5 ccs of antiformin have been transferred to the centrifuge tube, leaving 3 ccs of fæces and 15 ccs of antiformin in the bottle.

Mr A C Carter, Superintendent of Police, Purnea district, for the liberal facilities in conducting the investigation in their respective areas

My thanks are due to Captain P J Barraud of the Central Research Institute, for helping me in the mosquito work

Finally, I am thankful to my own staff, consisting of Captain K K Das, Civil Sub-Assistant Surgeon Babu Kulamoni Misra, and stenotypist Mr Gopal Krishna Chopra, for their assistance, zeal and loyal support

## II MATERIAL AND TECHNIQUE

### *Material*

The investigation has been tabulated under the following scheme The population of Bihar and Orissa has been classified into industrial, institutional and agricultural The agricultural group has further been divided according to the physiographical conditions of an area, situated at or above the sea-level This group of population might be either sparse or dense in any area The sparse conditions were mainly due to mountains, forests, marshes and unculturable areas The density was due to the regions being cultivated Cultivation depended upon the water-supply from the rains, canals and river inundations

The district areas of Bihar and Orissa were grouped according to the above scheme and the agricultural group of the population investigated accordingly

The examination was conducted usually during the night after the hours of 8 and also during the day

Fresh preparations of the peripheral blood were examined and the diagnosis of presence of microfilaria was made on the spot in many instances The cases in which microfilariae were found were classed as 'positive' and the rest as 'negative'

The cases selected for examination were males and females and of all ages The leading signs and symptoms were directed mostly to glandular, genital and terminal enlargements and to effusions of permanent or fugitive nature Along with the above signs a history of long duration and of fever was elicited

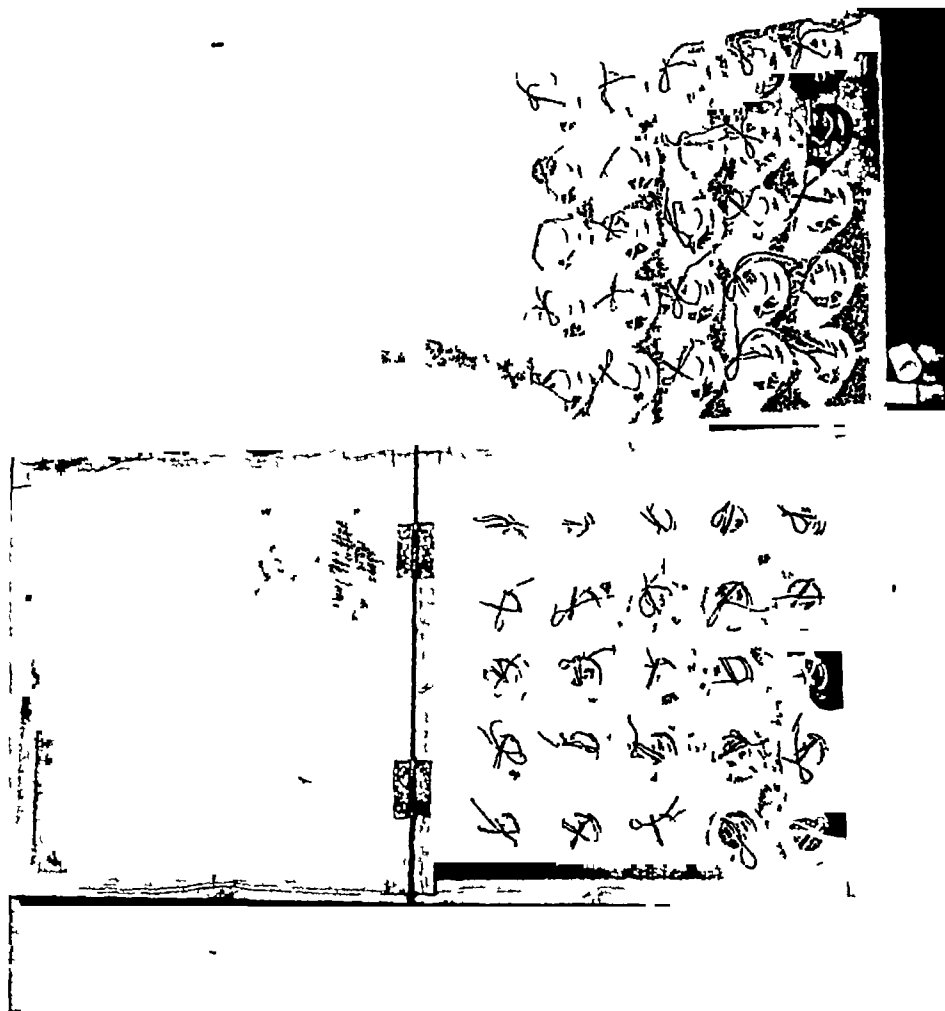
Thick smears were made from the cases showing microfilariae and the material was then brought to the field laboratory at Gaya, with a view to the study of the morphological details

The result of such a procedure has been the collection of filarial material in respect of the scheme Endemic foci have also been mapped out from the standpoint of epidemiology and much information has been gathered regarding the prevalent species of the parasite

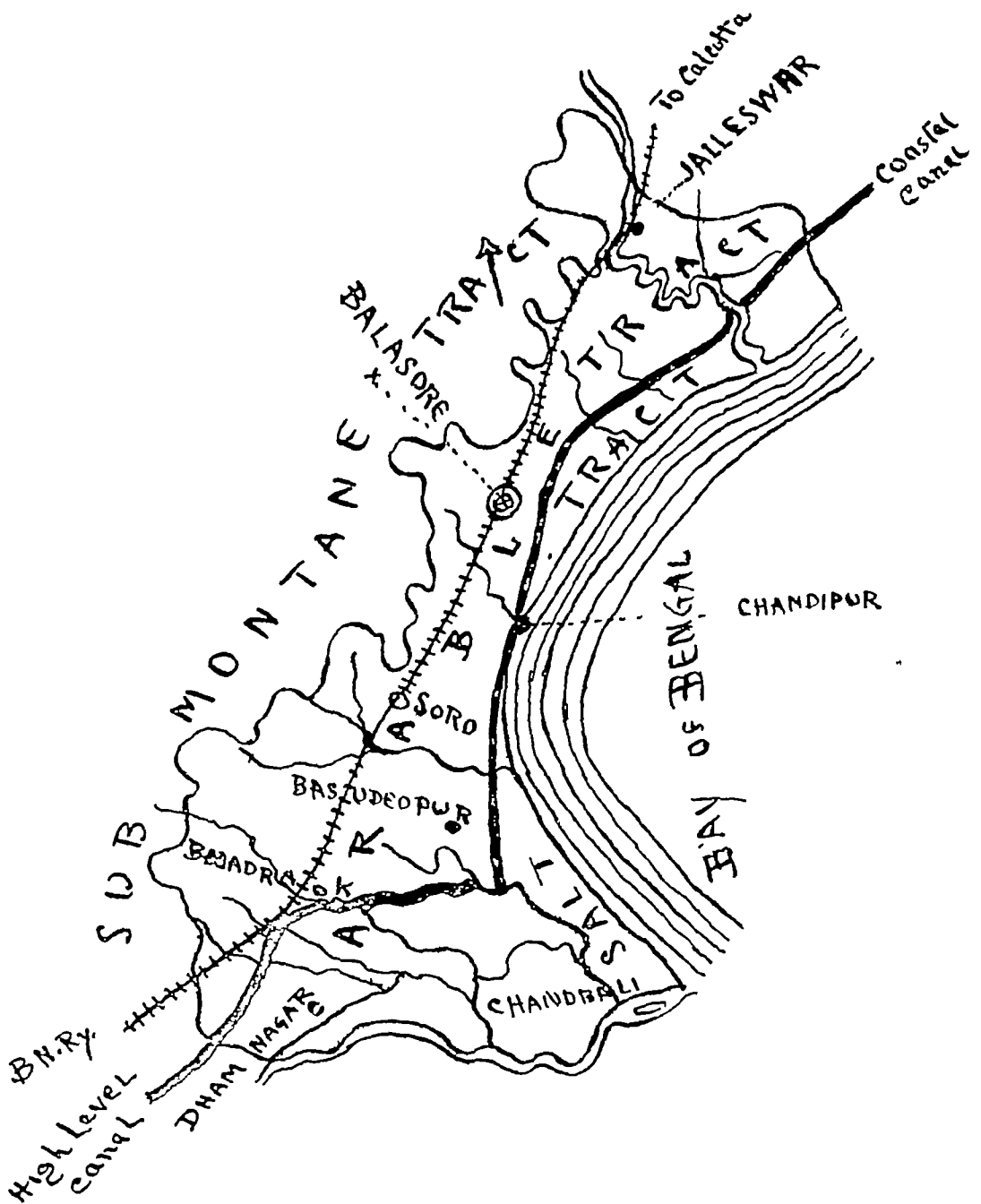
### TECHNIQUE

*Blood film*—The technique employed during this investigation was similar to that which has been followed hitherto (Korke, 1928), which consisted in taking a measured quantity of peripheral blood about 20 cmm, making thick smears, dehaemoglobinizing the blood and staining the films with a weak solution of Giemsa and hæmatoxylin on the approved lines.

# PLATE LIII



Photograph of a box with the tray removed standing beside it  
 Notepaper measures at foot of tray



Balasore District Area Scale about 1 inch to 18 miles

Mosquitoes prevalent *Tæmorhynchus (Mansonioides) uniformis*, *T. annuliferus*, *Culex epidesmus*

Predominant species of parasite *F. bancrofti* have been found in considerable number of cases but there is a variation of the type species which is under study

A RE-DESCRIPTION OF *WUCHERERIA BANCROFTI*  
(COBBOLD, 1877) WITH SPECIAL REFERENCE  
TO THE TAIL OF THE MALE.

BY

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THE material available for study consisted of two males, the anterior portion of a female less than two centimetres in length, and the middle part of a female. These worms were seen by a patient to issue from an abscess on the inner side of the upper arm, which had opened the day before and was discharging freely. They were rescued by Dr Sunder Rao, and handed to the writer for examination. Although the worms were dead when received, they were placed in normal saline for a time, and then transferred to 70 per cent alcohol, and they appeared to be in fairly good condition.

*Wuchereria bancrofti* has been variously described by different authors as having a smooth skin, or fine transverse striations, usually only visible in parts of the worm. In the present instance, the only portion that exhibited these markings was the pre-anal part of the ventral surface of the male.

The anterior portion of the female agrees with the usual descriptions. The head is slightly bulbar with a shallow mouth cavity not surrounded by papillæ or other appendages. The two rows of minute papillæ were present on the head. The vulva is situated at a distance of 0.695 mm from the anterior end. According to Walker (1914), who made careful measurements of several worms in the Philippine Islands, this dimension is 0.72 to 0.79 mm, and in the drawing by Yorke and Maplestone (1926), the vulva is about 0.84 mm from the anterior end, but these differences are of little importance, as they can be easily explained by shrinkage. The worm is about 0.23 mm in diameter at its widest part, this agrees fairly closely with Walker's measurements of 0.2268 mm.



Area, Manair (Patna district), density 600 and more The area is situated near-about the junction of the Sone with the Ganges and about 10 miles due west of Dinapur where the Sone canal empties into the Ganges

Total cases 104, positive 20, percentage infection 20

Mosquitoes prevalent *C fatigans* Parasite, *F bancrofti*

(d) Arcas, submontane, alluvial tract and partially cultivated mountain plateau

Hazaribagh area The district forms part of a chain of highland, sometimes a range of hills, sometimes a high cultivated plateau The plateau is elevated about 800 feet from the level of the Gaya plain (lower plateau), and reaches a general elevation of 1,300 feet and at the town of Hazaribagh about 2,000 feet above the sea-level (higher plateau)

The absence of water is the most striking feature in the scene of the lower plateau but on the higher plateau the country is open and the cultivation fairly extensive The surface is never level, there are no lakes and marshes in the district The average day temperature for the whole year is about 75°F and ranges between 40° and 107°F according to the season The average annual rainfall is about 50.52 inches The mean humidity is 51 The soil for the most part is inherently infertile and the density on the whole is 186 persons per square mile

Area, Barhi, situated in the Hazaribagh district, 23 miles north of the Hazaribagh town on the Grand Trunk Road Density 186 persons or less per square mile Total cases 34, positive nil

Mosquitoes prevalent, *C fatigans*, *A subpictus*

Area, Barakatha, 16 miles east of Barhi on the Grand Trunk Road Density like Barhi A jungle area Total cases 50, positive nil

Area, Chopra (Purnea district), situated at the north-east corner of the district practically on the border of Jalpaiguri and Darjeeling district, is a sub-montane alluvial tract Density 200 to 300 persons per square mile Total cases 67, positive nil

Mosquitoes prevalent *A fuliginosus*, *C fatigans*

(11) Filarial incidence in the town and institutional population in the areas situated at or above the sea-level

Town, Balasore, total population about 20,000 Population examined, adult males Total cases 31, positive 5, percentage infection 16

Hospital, Balasore, population examined, mixed adult, total cases 14, positive 2, percentage infection 14

Thana, Balasore, Police Force, Regular, strength about 400 (1905) population examined, adult males, total cases 80, positive 13, percentage infection 16

Thana, Balasore, Police Force, village chowkidars, strength about 2,000 (1905), population examined, adult males, agricultural Total cases 107, positive 26, percentage infection 24

Industrial School, Balasore, population examined, male, juvenile and adult, age, varying between 6 and 26 years Total cases 52, positive 9, percentage infection 17,

workers are due to the minute size of the worms, and the difficulty of examining them satisfactorily owing to the spiral turns of the tail, rather than to actual differences in structure

Leiper (1913) expresses the opinion that the number of papillæ may be subject to slight variation, but he describes, and in his somewhat diagrammatic drawing, figures fifteen pairs as being certainly present. He does not appear to have noted the two relatively large flap-like adanal papillæ, although these may be represented in his drawing by the papillæ lying at a slightly deeper level than the long row. He also neither mentions nor figures the definite transverse striations in front of the cloaca. The spicules in Leiper's material are essentially the same as those seen by the writer, but as they were not protruded from the cloaca in the latter case, it is not possible to say whether the fine terminal alæ described but not drawn by Leiper are present on the long spicule. The writer was also not able to make out the double tip of the short spicule as Leiper has drawn it, but this may be because it was lying over the gubernaculum in both his specimens. Leiper does not describe the gubernaculum, but his drawing of this structure is not very like the appearance seen by the writer.

Cruickshank and Wright (1914), in what is apparently a careful description of several well preserved specimens, describe two types of transverse cuticular striation, small closely set transverse lines and large annular constrictions. They suggest (probably correctly) that these markings may be due to shrinkage, especially as they say they are not present in all specimens. They note, however, the presence of fine corrugation on the pre-anal portion of the male. They describe and figure eleven pairs of papillæ in one male and nine pairs in another. They also describe two dissimilar spicules, much the same as those seen by other workers, but they state that the short spicule ends in a fine lash like the long one. This has not been seen by anyone else.

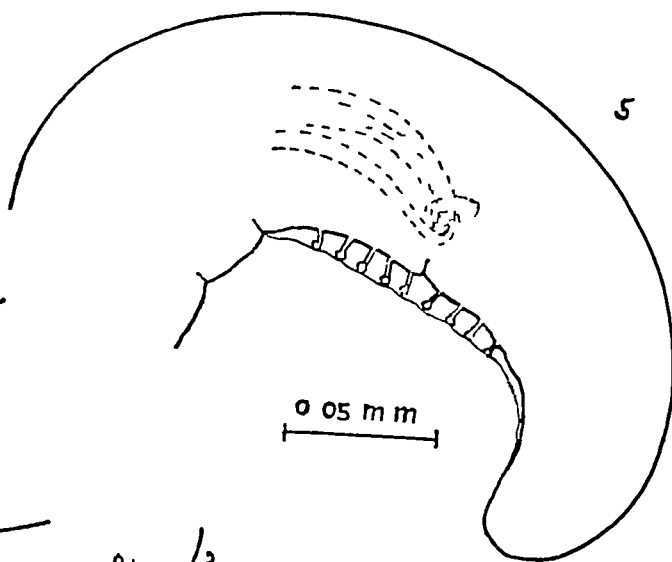
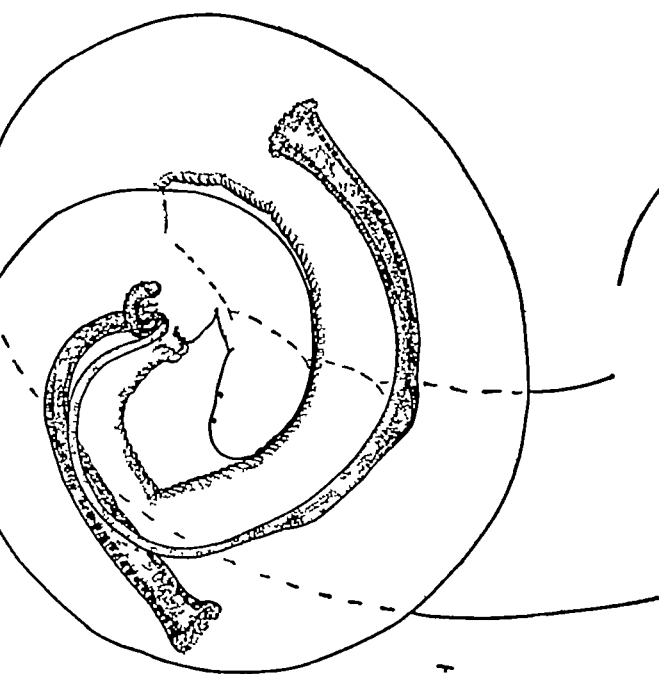
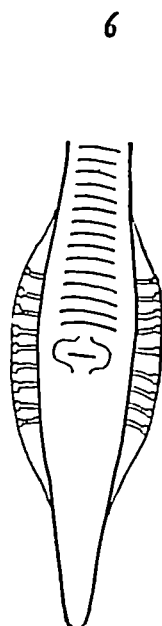
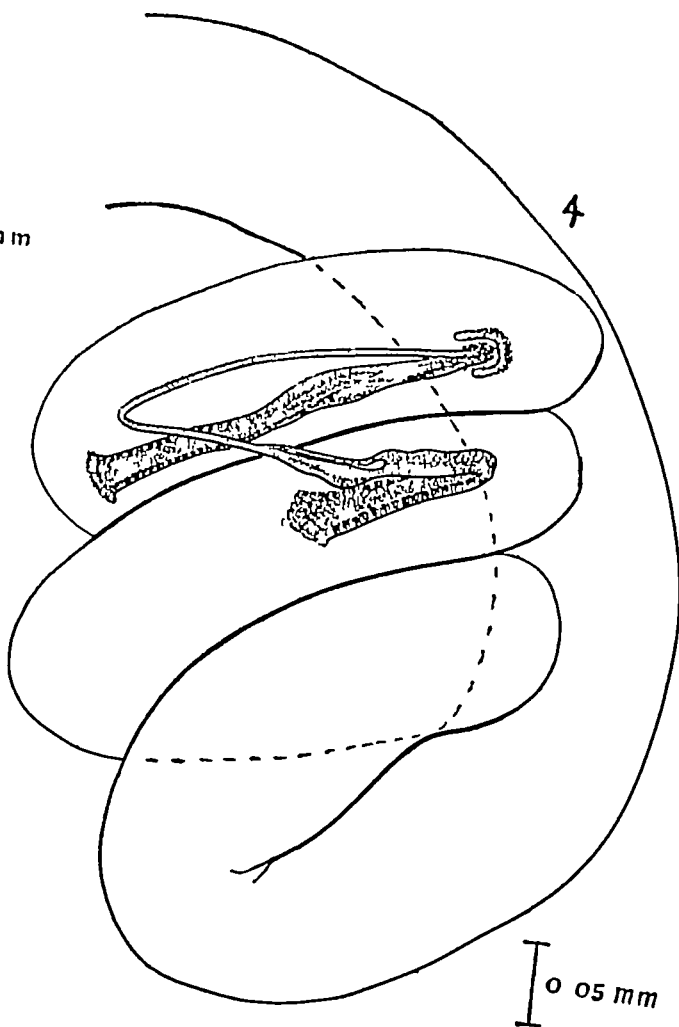
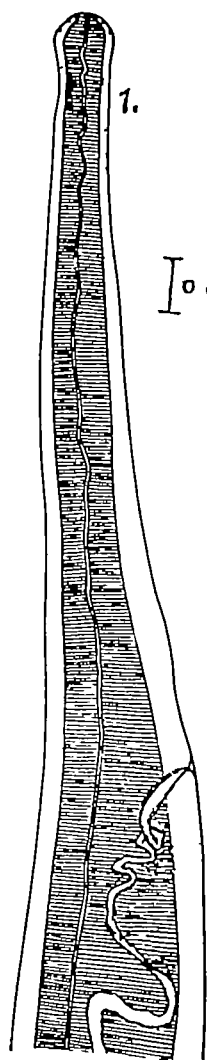
Walker (1914) states that there are three pairs of post-anal and thirty-two pairs of pre-anal papillæ, which are very small and difficult to see. But his drawings are very small and unconvincing as they show no papillæ at all. It is possible that he mistook the corrugation of the skin in this position, as papillæ. He also says the female exhibits very fine striations, these are not present throughout the length of the worm, but only in parts.

Rivas (1920) says the cloaca of the male opens between two prominent papillæ, and he quotes Looss as saying that there are three pairs of pre-anal papillæ.

Consideration of the above statements by various authors shows that there is considerable disagreement on the minute anatomy of the tail of the male. But prolonged and repeated examination of the two specimens available to the writer, makes it clear that an exact description of the worm is a very difficult matter. For example, both specimens were examined on several occasions in the cleared state, from many different angles, with different powers of the microscope, and with varying degrees of illumination, and all that could be made out with certainty were two pairs of minute papillæ towards the tip of the tail, and a single



# PLATE LIV



16-3

TABLE I-B  
*Showing Filarial Incidence in the areas of Bihar and Orissa*  
 Cases not showing Microfilariae in Peripheral Blood

Serial Nos	Areas	CASES WITH CLINICAL SIGNS AND SYMPTOMS—ANALYSIS										Cases with- out signs and symptoms	REMARKS
		Total cases	Duration (years) Illness	History, fever	ENLARGEMENTS				EFFUSIONS				
					Glandular	Scrotal	Testicular	EXTREMITIES		Tunica vaginalis	Fugative nature		
								Upper	Lower				
1	At sea-level— Bhadrak	17	1—12	2				2	80	15		516	
2	Soro	80	1—20									22	
3	Jalleswar	8	2—12	2					5	1		74	
4	Coastal canal— Chandipur	2	1	1	1				2			29	
5	Basudebpur	23	3—10	10	1				6	8		54	
6	Above sea-level— Bhalua	2	8—20							2		76	
7	Sherghati	9	1 1/2—15	1			3			5		44	
8	Kishanganj	1								1		110	

# NOTES ON SOME HÆMATOLOGICAL AND SEROLOGICAL INVESTIGATIONS IN LEPROSY

BY

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AND

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THE examination of the blood in leprosy, apart altogether from its investigation with a view to discovering a 'specific test' for the presence of this disease, has engaged the attention of workers in different parts of the world. Studies on the cellular elements of the blood in leprosy have been reported by Pringault (1912), Lagane and Colombier (1913), Knapp (1915), Sadı de Buen (1916), Leger (1921), Mitsuda (1923), Paldrock (1925), Bargehr (1926), Henderson (1927) and others. The improvements which have taken place in blood chemistry technique in recent years have prompted investigations on the chemical constitution of the blood in leprosy. A wide range of substances including the inorganic constituents Ca, Mg, P, the chlorides, cholesterol, non-protein nitrogen and sugar have received treatment at the hands of Underhill et alia (1920) Marchand (1922), Boulay and Leger (1922), (a), (b), Balbi (1925), Paras (1926), (1927), Concepcion and Salcedo (1926) Lemann et alia (1927) and Boyd and Roy (1928). The recent impetus given to the study of the sedimentation of erythrocytes has resulted in enquiries on this subject (so far as it pertains to leprosy) on the part of Puxeddu (1924), Gilbert et alia (1926), Landeiro (1926), Labernadie and André (1927) Iturbe (1927) and more recently Muir (1928).

TABLE II.  
*Showing Incidence of Filariasis in the areas investigated*

Areas	Total cases	Total positive	CASES				EXAMINATIONS					
			With clinical signs		Without signs		Day		Night		Combined	
			Total	Positive	Total	Positive	Pos	Neg	Pos	Neg	Pos	Neg
Bhadrak	610	77	22	5	588	72	5	121	72	412		.
Soro	117	15	83	3	34	12	1	51	14	51		.
Jalleswar	106	24	8		98	24			24	82		
Chandipur	42	11	2		40	11			11	31		
Basudebpur	108	31	27	4	81	27			31	77		.
Bhalua	85	7	4	2	81	5	1		7	78	1	
Sherghati	61	8	10	1	51	7			8	53		
Kishanganj	123	12	1		122	12			12	111		
Arwal	110	13	24	6	86	7		7	13	97	.	7
Paliganj	113	20	32	11	81	9	7	4	20	93	7	4
Manaur	104	20	16	6	88	14		5	20	84	.	5

TABLE I—(contd)

'B' type comprising 'B <sub>1</sub> ' 'B <sub>2</sub> ' and 'B <sub>3</sub> ' cases	Specific gravity of blood
L B	1053
R	1048
A	1057
S S	1056
R. M	1052
S	1047
N	1049
I	1052
S C G	1062
R. R P	1054
G M	1054
A R	1051
B B	1050
B	1047
M M	1053
I Q	1054
D K.	1053
G	1050
M L	1056
D	1048
K. M P	1054

Group average—1052.

Rogers (1913) gives the average specific gravity of blood in the healthy Indian as 1054. The type and stage of leprosy *per se* appears therefore to have little effect on the specific gravity of the blood. Advanced types appear if anything, however, to be associated with a slight lowering of specific gravity.

Table II shows the number of cases in each group falling below the group average together with the lowest individual reading in the group.

TABLE II

Group	Total cases	Number of cases below group average	Percentage below group average of whole group	Lowest Reading
A	9	3	33.3	1050
B	21	8	37.1	1047



The submontane and the plateau areas investigated so far have given negative results

The arable areas above the sea coast, as for instance the Patna and Gaya districts, are infected next in order, Patna showing 18 per cent and Gaya 11 per cent of infection

The Sone canal commences at Barun in the Gaya district, traverses the Gaya and Patna districts north-eastwards for about 80 miles and opens into the Ganges near Dinapur east of Manair. In the canal area, three areas were investigated in the Gaya district, viz, Barun, Daudnagar and Arwal and two areas, viz, Paliganj and Manair in the Patna district

These areas are situated equidistant from each other and are given in their order from the commencement of the canal to its fall into the Ganges

The percentage of infection is 2 at Barun, 10 at Daudnagar (Korke, 1928), 12 at Arwal, 18 at Paliganj and 20 at Manair

The results show that the incidence of infection rises as one traverses the canal along its course

(iv) (b) Results of blood examination at different hours in groups of the agricultural population of the same area

The observations were systematically made in the Bhadrak and Soro areas of the Balasore district. A large section of the agricultural population from the villages of the same area was attending the Survey and Settlement Offices and examination of the blood was continued from morning to night. The results are tabulated under Table III

TABLE III

*Result of Blood Examination at 'different hours' in Groups of Agricultural Population of the same areas, Balasore District*

Hours	BHADRAK			SORO		
	Total cases	Total positive	Percentage	Total cases	Total positive	Percentage
8—10	13	1	7.5			
10—12	43	1	2			
12—14	3					
14—16	45	2	4			
16—18	22	1	5	52	1	2
18—20	172	24	14	13	2	15
20—23	312	48	15	52	12	23

It will be seen from the above table that the blood examination conducted during the day time at Bhadrak gave a 4 per cent positive result while the examination conducted during the night gave 16 per cent positive result. In the Soro

NaCl concentration This also is within the normal range and any individual variations fell within the limits of experimental error

### (3) *Surface tension of Blood Serum*

For the estimation of this property we employed a Traube Stalagmometer and in order to ensure uniformity of results particular attention was paid to the following points —

- (a) Cleanliness of the instrument The presence of foreign matter and particularly of grease, profoundly alters the results The instrument was carefully cleaned out with distilled water, absolute alcohol and ether and finally placed in the hot air steriliser, protected from dust, for  $\frac{1}{2}$  an hour
- (b) Uniformity in the size of drops Marked differences in readings may result from differences in the size of drops To eliminate this source of error so far as possible, the instrument was suspended vertically, its position being checked by the plumb line The mouth of the stalagmometer was also carefully wiped with a clean cloth immediately prior to counting lest variations should arise consequent on partial drying of the column of serum immediately above the dropping orifice
- (c) Air bubbles were rigidly excluded from the column of fluid
- (d) Successive estimations were carried out at the same temperature —  $37^{\circ}\text{C}$
- (e) All active treatment had been suspended for at least one week in the patients selected for experiment, lest alterations in surface tension might be caused by this factor

*Method* — In the absence of a regular thermostat we employed a bacteriological incubator regulated at  $37^{\circ}\text{C}$  The stalagmometer was suspended vertically in the incubator and attached to its upper end was a long piece of close fitting rubber tubing which passed through a cork filling the thermometer opening in the roof of the incubator The other end of the tubing was clamped and a centigrade thermometer suspended inside the incubator

About 10 ccs of blood having been drawn from the median basilic vein into a sterile test-tube, the serum was separated from the clot and placed in a small clean glass vessel inside the incubator Serum was then drawn up into the stalagmometer by suction through the rubber tubing until the upper level of the column of serum was just above the top mark on the stalagmometer The column was then allowed to fall until the meniscus of the fluid reached this mark after which the rubber tubing was clamped The vertical position of the instrument was checked and the column of fluid carefully inspected for air bubbles The temperature having become steady at  $37^{\circ}\text{C}$  the dropping orifice of the instrument was carefully wiped with a clean cloth, the position of the instrument again checked, the rubber tubing unclamped and the drops counted as the fluid

TABLE IV.  
Showing Age and Sex Incidence

AREAS	GAYA DISTRICT.				PATNA DISTRICT				PURNA DISTRICT (NO FEMALES)				BALASORE DISTRICT			
	Male		Female.		Male.		Female		Male		Male		Male		Female	
	Cases		Cases		Cases		Cases		Cases		Cases		Cases		Cases	
	Total	Positive.	Total	Positive.	Total	Positive	Total	Positive	Total	Positive	Total	Positive	Total	Pos	Total	Pos
0—10	5	1	3	..	1				2		48	2	23	2		2
10—15	30	.	4		22	5	1		2	1	133	14	26	5		5
15—20	51	3	3		22	5			18	2	158	30	17	1		1
20—25	48	4	1		25	5	1		25	2	155	16	10	1		1
25—30	69	6		.	30	7	2	1	35	3	312	57	15	1		1
30—35	64	12	.		25	5	1		43		149	32	6	2		
35—40	54	8	2		26	4	3		23		142	26	3	.		.
40—45	23	5		..	20	3	1		17	3	69	11	1			
45—50	15	1		.	18	2	1	.	9		93	12	3			
50—above	8	2	..	..	18	3			16	1	83	22	4			
Total	367	42	13	..	207	39	10	1	190	12	1,342	222	108			12

absolute values are desired, a point of importance emphasised in a recent paper by Johlin (1928) We were not concerned, however, with absolute values our sole aim was to determine (a) whether there was any difference between the surface tension of leprotic and normal sera tested under approximately identical conditions, (b) whether any alterations observed in the surface tension could be related to the speed of sedimentation of the erythrocytes From the figures given above it seems justifiable to make the following statements

- (1) The surface tension of leprous sera is on the whole somewhat lower than that of normal sera tested under approximately identical conditions but the difference is not one which can be regarded as having any clinical significance
- (2) The surface tensions of sera from cases in the quiescent and reactionary phases of leprosy are approximately the same
- (3) There tends to be a fall in surface tension with acceleration of the sedimentation rate of the erythrocytes

#### SUMMARY AND CONCLUSIONS

1 Observations have been made on the specific gravity of the blood, the fragility of the red blood cells and the surface tension of the serum in leprosy cases

2 The type and stage of leprosy *per se* appear to have little influence on any of these phenomena

3 Cases of leprosy showing increase in the rate of sedimentation of erythrocytes tend to show also a fall in the specific gravity of the blood and in the surface tension of the serum

A full bibliography is appended including all the papers of importance dealing with physical, chemical and hæmatological investigations in leprosy

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A number of species of mosquitoes under *Culex* and *Anopheles* have been described in the areas investigated but apart from the developmental stages of *bancrofti* in *C. fatigans*, I am yet unable to say whether such developmental stages are found in the other species. The possibilities are that in the Balasore district area species of *Culex* and species described under *Tanorhynchus* act as intermediary hosts of *bancrofti*.

#### IV DISCUSSION OF RESULTS

The following points present themselves for discussion

##### 1 *The incidence of filariasis in relation to the physiographical conditions*

I have pointed out before (Korke, 1928), that to arrive at a true situation of the filarial condition of an area, one has to consider the sequence of events which goes to determine the economic aspect of the agricultural section of the population of the area. The economic aspect is influenced by the type of the soil, by the physical features of the country, temperature, and water available for the irrigation. These factors encourage the agricultural section of the population to grow the crop and adapt the soil to a particular type of cultivation.

Water, whether derived from rains, rivers (inundations), canals, wells or artificial reservoirs, is the very basis of agriculture and also that of the breeding of mosquitoes. This condition also is responsible for the upkeep of the species of mosquitoes in luxuriance.

Breeding and upkeep of the species, which acts as an intermediary host of filaria lead to the preservation of *bancrofti* more or less on a permanent footing. Once the footing is gained the areas become a permanent *endemic centre* of filariasis especially where the density of persons per square mile is considerable and supported by the insanitary conditions of the villages.

Taking in this way a very broad outlook of factors in the epidemiology of filaria, the results seem to show that the incidence is proportional to the factors discussed.

A type area (Balasore), investigated at the sea-level, shows that the area is riddled with rivers which flow into the sea, possesses a system of canals which traverse the length of the district, has a configuration which tends to bring on heavy river-inundations during the monsoons, retains sub-soil moisture which readily provides water in shallow excavations, (hence the preponderance of wells and artificial reservoirs), and lastly has a rainfall sufficient for the agricultural needs of the people.

Reading these conditions in association with the results, the area shows the *highest filarial incidence* in the agricultural section of the population that has hitherto been investigated.

Further, these observations are corroborated in the areas where plentiful supply of water is available for the irrigation purposes. The Sone canal area serves as the type. As one proceeds along its course, one finds the incidence of infection *rising* from Gaya through Patna towards the Ganges. The result may partly be attributed to the density of population which is higher in the Patna

# OBSERVATIONS ON FILARIASIS IN SOME AREAS IN BRITISH INDIA

## Part III.

BY

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- II MATERIAL AND TECHNIQUE
- III RESULTS OBTAINED IN FIELD STUDIES
  - (A) Filarial Incidence in the areas of Bihar and Orissa
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    - (i) Human Phase
    - (ii) Mosquito Phase
- IV DISCUSSION OF RESULTS
- V CONCLUSIONS

## I INTRODUCTION

THE investigation into filariasis was undertaken under the auspices of the Indian Research Fund Association, Simla, and covered a period from April to July 1928. Gaya was made the centre of the inquiry.

The inquiry was conducted on similar lines as have been published before (Korke, 1928). Briefly stated, the object of the inquiry was to determine the species of microfilaria prevalent in the endemic areas in relation to the physical characters of the district, to observe the early clinical features associated with such species, to study systematically the morphology of the microfilaria and lastly to investigate the life-cycle of the parasite in the invertebrate host.

The observations were made in the district areas of the province of Bihar and Orissa.

I am greatly indebted to the Government of Bihar and Orissa for their generous help, and to Captain R P Ward, I C S, Collector and Magistrate of the Balasore district, Mr I C McNally, Superintendent of Police, Balasore, and

peripheral blood for nearly three hours between 9 and 12, begin to appear in progressive numbers for twelve hours between midday and midnight and reach the maximum figures for six hours between 24 and 6 (Korke, 1928) This observation was made on a single case and to confirm this a large section of agricultural population belonging to the same area was examined in batches from eight in the morning till eight in the evening, in the Balasore district It was not possible to continue the observations further during the night without prejudice to the inquiry The results confirm that 4 to 11 times better results are obtained where the examination is conducted after 6 p.m. and where the diagnosis for filariasis is to be made by the finding of microfilaria in blood

## V CONCLUSIONS

1 There is a considerable prevalence of filariasis in the Bihar and Orissa area, 14 per cent of the general population shows the presence of microfilaria in blood

2 The evidence of filariasis is uniform throughout in an area cultivated chiefly with paddy

3 The incidence of filariasis was found to be the highest in an area situated at the sea-level and lowest or nothing in the submontane and plateau area In the Gangetic valley the areas of Gaya and Patna appear to be uniformly infected and the infection appears to be the greatest where plenty of water is available for irrigation

4 15 per cent of cases *showed microfilaria* in the peripheral blood where filariasis *was suspected* on clinical grounds 12 per cent of cases *did not show microfilaria* in blood where it *was suspected* on clinical grounds

5 14 per cent of cases *showed microfilaria* in the peripheral blood where filariasis *was not suspected* on clinical grounds (total cases 2,036, positive 278)

6 17 per cent of males, 11 per cent of females and 12 per cent of juvenile females are infected in the Balasore district 5, 22, 14 and 14 per cent of juvenile males are infected in the districts of Gaya, Patna, Purnea and Balasore, respectively

7 The microfilariae were detected most readily during the night time

8 A notable clinical feature was the affection of the genitals in the area above the sea-level and affection of the lower extremity in the areas at the sea-level

9 The predominant species was *F. bancrofti*

10 The developmental stages of *bancrofti* were observed from the time of the ingestion of the parasite by the mosquito up to the time when the parasite had infected the proboscis The prevalence of *Culex fatigans* was marked in all the areas investigated but in addition *C. vishnui*, *C. epidesmus*, *T. uniformis* and *T. annuliferus* were present in large numbers where the incidence in filariasis was found to be the highest (Balasore area)

Wherever *intra vitam* staining was resorted to, it was done by Azure II in normal saline, strength 1 in 3,000

*Mosquito*—With a view to study the life-cycle in the invertebrate host mosquitoes were caught in nature from the houses situated in known endemic areas. They were kept alive in the laboratory in accordance with the technique described before, (Korke, 1928)

### III RESULTS OBTAINED IN FIELD STUDIES

#### (A) *Filarial Incidence in the areas of Bihar and Orissa*

(i) *Filarial incidence in the agricultural group of the population in accordance with the physiographical conditions*

(a) *Areas at the sea-level*, Balasore district area. The area forms the south-east sea-board of the province, is about 2,000 square miles with a population of 1,100,000, is divided into three longitudinal strips of land, 'salt tract,' arable tract and submontane tract, from east to west respectively. Salt tract about 6 to 10 miles in breadth, arable tract 10 to 40 miles and the submontane tract forms the western boundary of the district. The coastal canal separates the salt from the arable tract, and the latter is dead-level of rice-fields. Temperature ranges between 74° and 98°F according to season. Rainfall 60 inches, humidity 79 to 89 per cent, April-May to August. Cultivation depends upon the rainfall, canal and river inundations. The river system is from north to south (*see* Map on page 698)

Area, Bhadrak, arable, density 500 to 600 persons per square mile. Night examinations, total cases 484, positive 72, percentage infection 16, Day examinations, total cases 126, positive 5, percentage infection 4

Mosquitoes prevalent *Culex bitamorphynchus*, *C. vishnu*, *C. fatigans*, *Armigeres obturbans*, *Aedes (Stegomyia) vittatus*, *Tamorphynchus (Mansonioides) annuliferus*

Area, Soro, arable, density 500 to 600 persons per square mile. Night examinations, total cases 65, positive 14, percentage infection 22. Day examinations, total cases 52, positive 1, percentage infection 2

Mosquitoes prevalent *Tamorphynchus (Mansonioides) annuliferus*, *Tamorphynchus (Mansonioides) uniformis*, *Culex vishnu*

Area, Jalleswar, arable, density 500 to 600 persons per square mile. Total cases 106, positive 24, percentage infection 23

Mosquitoes prevalent, *Culex fatigans*, *C. bitamorphynchus*, *C. vishnu*, *C. whitmorei*, *C. epidesmus*, *Lutzia fuscana*, *Tamorphynchus (Mansonioides) uniformis*

Area, Chandipur, coastal canal, density 300 to 400 persons per square mile. Total cases 42, positive 11, percentage infection 26

Mosquitoes prevalent *Culex fatigans*

Area, Basudebpur, coastal canal, density 500 to 600 persons per square mile. Total cases 108, positive 31, percentage infection 29,



NOTES ON SOME INDIAN SPECIES OF THE GENUS  
*PHLEBOTOMUS*.

Part XXIV.

*PHLEBOTOMUS BARRAUDI* N SP

BY

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(*Malaria Survey of India*)

[Received for publication, September 22, 1928]

IN a collection of 'sandflies' sent by Captain P J Barraud, r r s, from Golaghat, Assam, were found a number of specimens of a species of the 'recumbent-haired group,' which were at the time provisionally diagnosed as *P minutus* var Since the value of the morphology of the buccal and pharyngeal armatures was recognised in the identification of the members of this group, it has been found that the armatures of these Assam specimens differed from those of any of the other species described from India It is proposed that this new species be named *Phlebotomus barraudi* after Captain P J Barraud, who so kindly sent me the specimens

*Material*

The original specimens of this species were collected at Golaghat, Assam, in a horse stable, but a number of other specimens have been found in a collection containing *P argentipes* and *P shortti* from the same locality All the specimens so far examined were captured in November, 1924

*Phlebotomus barraudi* n sp (♀).

The insects were very dark grey in colour, some showing a brownish tinge The abdominal hairs were sleek and recumbent on the dorsum, while on the venter they had a more ruffled appearance and were inclined to be semi-recumbent These hairs were dark brown with a greyish coloration in some lights They were more numerous than those usually seen in *P babu*

(b) *Areas above the sea-level*, arable alluvial tracts

Density 200 to 300 persons per square mile

Area, Bhalua (Gaya district), 36 miles south-east of Gaya, a jungle area, well watered and at the commencement of the Hazaribagh plateau

Total cases 85, positive 7, percentage infection 8

Mosquitoes prevalent, *C fatigans*, *C (culicomyia) pallidothorax*, *C bitamorphynchus*, *C vishnu*, *C (culicomyia) pullus*, *Lutzia fuscana*, *Anopheles culicifacies*, *Anopheles subpictus*

Parasite, *F bancrofti*

Area, Sherghati (Gaya district), enclosed between two arms of the river Morhar, a river which carries a considerable body of water during the monsoons. The area is situated at the commencement of the northern slopes of the Hazaribagh hills

Total cases 61, positive 8, percentage infection 13

Parasite, *F bancrofti*

Area, Kishanganj (Purnea district) The district lies towards the eastern extremity of the Gangetic plain, is hemmed in by the Ganges on the south, and the Nepal hills on the north. Some of the areas possess the features characteristic of a submontane alluvial tract. The surface is almost a dead-level and traversed by a number of rivers and streams. For practical purposes, the district may be divided into two portions, by a line drawn diagonally north-west to the south-east corner. The country to the east being drained by the Mahananda and that to the west by the Koshi. In the east the soil is fertile and loamy and rich in crops, rice and jute. The general features are like those of Bengal.

The climate of Purnea may be described as mean between that of Bihar and Bengal. The mean temperature ranges between 62 and 48 in January and 75 and 84 in March and May. Highest mean maximum temperature is 95°F in April. The average annual rainfall is 72.5 inches.

Area, Kishanganj, density 400 to 500 persons per square mile. Total cases 123, positive 12, percentage infection 10.

Mosquitoes prevalent, *C fatigans*, Parasite, *F bancrofti*

(c) *Areas above the sea-level*, arable, the Sone canal area

Area, Arwal (Gaya district), density 600 and more. Situated about 40 miles north of Barun which is the commencement of the Sone canal.

Total cases 110, positive 13, percentage infection 12

Mosquitoes prevalent, *C fatigans*, *Anopheles culicifacies*

Parasite, *F bancrofti*

Area, Paliganj (Patna district), about 20 miles north of Arwal. Density 600 and more.

Total cases 113, positive 20, percentage infection 18

Mosquitoes prevalent *C fatigans*, *Aedes (Stegomyia) albopictus*; *Anopheles culicifacies*

Parasite *F bancrofti*

J, MR

TABLE I—(concluded)

Structure	LENGTH IN MM OF SPECIMENS NUMBERS —				REMARKS, RELATIVE LENGTHS, ETC. †
	1*	2	3	4	
Segment 1	0·042	0·042	0·036	0·042	Formula 1,2,3,4,5
Segment 2	0·093	0·087	0·084	0·087	Relative lengths 2·9, 6·3, 8·8, 10, 22·4
Segment 3	0·132	0·120	0·117	0·120	$\frac{P}{E} = 3·26-3·90,$
Palp Segment 4	0·147	0·135	0·132	0·132	$=\frac{1}{2}$ length palp
Segment 5	0·360	0·240	0·264	0·288	
Total length	0·774	0·624	0·633	0·669	$=0·45-0·54 \times \text{antenna},$ $0·38-0·46 \times \text{wing}$
Length	1·670	1·643	1·570	1·643	$3·66-3·93 \times \text{breadth},$ $0·67-0·69 \times \text{leg}$
Breadth	0·430	0·430	0·430	0·414	$\frac{\alpha}{\beta} = 0·86-1·05 \frac{\beta}{\gamma} = 0·90-1·22$
α	0·278	0·278	0·257	0·264	$\frac{\beta}{\gamma}$
β	0·300	0·293	0·271	0·271	$\frac{\alpha}{\gamma} = 0·88-1·11 \frac{\delta}{\alpha} = 0·31-0·52$
γ	0·257	0·300	0·257	0·300	$\frac{\delta}{\alpha}$
δ	0·120	0·135	0·100	0·107	$\frac{\alpha}{\epsilon} = 0·66-0·72 \frac{\beta}{\epsilon} = 0·67-0·78$
ε	0·414	0·407	0·371	0·400	$\frac{\theta}{\epsilon} = 1·91-2·02 \frac{\alpha + \beta}{\theta} = 0·67-0·71$
θ	0·828	0·814	0·757	0·800	
π	0·100	0·100	0·071	0·078	
Femur	0·685	0·671	0·628	0·643	$\frac{\text{Wing length}}{\theta} = 1·90-2·08$
Tibia	0·800	0·814	0·728	0·828	$=\frac{1}{2}$ length leg
Tarsus, seg 1	0·371	0·385	0·343	0·378	
Tarsus, segs 2—5	0·585	0·571	0·557	0·571	$=\frac{1}{4}$ length leg
Total length	2·43	2·43	2·26	2·42	(not including coxa and trochanter)

\* Type female

† Data from 9 specimens

The total *length* of the insects varied from 2·0 to 2·36 mm in mounted specimens. The length averaged about 0·88 times that of the hind leg and 1·33 that of the wing.

Zilla School, Balasore, population examined, juvenile, male, age, varying between 8 and 20 years Total cases 75, positive 10, percentage infection 13

Mission School, Balasore, population examined, juvenile male, age, varying from 5 to 18 years Total cases 31, positive 3, percentage infection 10

Mission Orphanage, Balasore, population examined, juvenile and adult, female, age, varying from 7 to 21 years Total cases 77, positive 8, percentage infection 10

Mosquitoes prevalent in the Balasore town area

*C fatigans*, *C vishnu*, *T (Mansonioides) annuliferus*, *T (Mansonioides) uniformis*, *Aedes (Skusea) micropterus*

Species of parasite, *vide* remarks under area Basudebpur (i), (a)

Gaya town, population about 60,000 Total cases 24, positive 3, percentage infection 13

Police Force, Gaya, Regular, strength about 800 (1919) Total cases 100, positive 11, percentage infection 11

Mosquitoes prevalent *C fatigans* Parasite, *F bancrofti*

(iii) Clinical signs and symptoms in the population investigated

The cases are divided into two groups, A, in which microfilariæ were detected in the peripheral blood and B, in which they were not detected The results are shown under two tables, Table I-A and Table I-B Both the tables show that the signs and symptoms common and present in a very large number of cases are the duration of illness, the affection of tunica vaginalis and the history of fever

Affections of the scrotum, testicle and upper extremity are present in low percentages

Affections of glands, especially of the inguinal, are 11 times more in cases showing microfilariæ while affections of the leg are three times more in cases not showing microfilariæ In other words, elephantiasis of the lower extremity is more associated with the absence of microfilaria in the peripheral blood and the enlargement of the glands with the presence of microfilariæ in the peripheral blood

Another feature is that only 15 per cent of the population showing the parasite in the peripheral blood show clinical signs and symptoms and 85 per cent do not show such manifestations The results are practically similar in cases not showing the parasite in the peripheral blood, viz, 12 and 88 per cent respectively

(iv) (a) Incidence of filariasis in the areas investigated

The results are tabulated under the form of a table (Table II)

The figures in the percentage infection are given under each area, *vide* items (i) and (ii) They show that the prevalence of filariasis is greater in the district bordering the sea, viz, the Balasore district of the Orissa division In the Balasore district itself, the 'saline' or sea-coast tract appears to be more infected than the central arable tract, example, Chandipur and Basudebpur 26 and 29 per cent respectively

*P. malabaricus*, *P. sylvestris*, *P. minutus*, *P. babu*, *P. shortti* and *P. squamipennis*

The wing venation resembles that of *P. montanus*, but the wing of the latter is narrower, the ratio length over breadth being greater than 4. The buccal cavity also shows a different morphology (cf. Sinton, 1927, Plate VIII, fig. 10).

The buccal cavity and pharynx show some resemblance to those of *P. africanus* (cf. Adler and Theodor, 1927, Plate IX, fig. 5) but a closer examination shows many differences. The wing venation is also different.

The African species, *P. simillimus* Newst., resembles this species both in wing venation and the long IIIrd antennal segment. The latter is, however, much longer than the same segment in *P. barraudi*. The relative lengths of the segments of the palps of four African specimens examined by me averaged 2.6, 4.9, 7.5, 10, 10.6 as compared with 2.6, 6.3, 8.8, 10, 22.4 in *P. barraudi*. The wing of *P. simillimus* is relatively broader. *P. simillimus* is also a larger species.

*Phlebotomus barraudi* n. sp. (♂)

The insect is dark brown in colour and has more or less the same general appearance as the female. The abdominal hairs are more numerous than those usually seen in males of such recumbent hairs species as *P. babu*, *P. shortti*, etc.

*Appearances in Stained and Mounted Specimens*

The measurements of the different parts of the body in four specimens are given in Table II and the ratios, etc., determined from eight specimens are also shown.

TABLE II  
*Phlebotomus barraudi*, N. SP. (♂)

Structure	LENGTH IN MM. OF SPECIMENS NUMBERS —				REMARKS, RELATIVE LENGTHS, ETC. †
	*1	2	3	4	
Clypeus and head	0.328	0.330	0.300	0.314	
Thorax	0.514	0.528	0.457	0.500	
Abdomen proper	0.857	1.076	0.857	0.814	
Sup. clasper, seg. 1	0.200	0.214	0.198	0.190	
Total length	1.90	2.14	1.81	1.82	
					=1.26–1.65 × wing, 0.84–1.02 × leg
Labium	0.170	0.180	0.170	0.170	extends beyond end of antenna seg. III
Epipharynx	0.150	0.171	0.156	0.150	

\* Type male

† Data from 8 specimens

	Sone canal—	6	1-10	1	2	.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg	
9	Arwal	.	1-10	1	2	.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg	
10	Paligany	.	1-4	1	6	1	1	6	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg
11	Manair	6	1-20	3	3	1	1	3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg
	Submontane and plateau—																															
12	Barhi	.	.																													
13	Barakatha	.	.																													
14	Chopra	.	.																													
	Towns, etc.—																															
15	Balasore	2	1-12	1	1		1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg
16	Hospital	2	1-5	4	1	.	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg
17	Police, Regular	4	1-4	4	1	.	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg
18	Police, chowkidars	3	4-6	3	1	.	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg
19	School, Industrial																															
20	School, Zilla																															
21	School Mission																															
22	Orphanage Mission																															
23	Town, Gaya	1	11	1	1		1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	One case eczema leg
24	Police, Regular, Gaya																															
	Total	50	87	28	34	1	2	4	7	16	27	44	71	108	165	242	363	544	826	1249	1873	2812	4217	6326	9489	14233	21352	32135	48168	71753	107411	241411
	PERCENTAGE	15	87	28	34	2	4	7	14	32	63	108	171	261	392	588	882	1323	2014	3021	4532	6798	10197	15296	22944	34416	51624	77436	116154	174231	261346	391719

TABLE II—(concl'd)

Structure.	LENGTH IN MM OF SPECIMENS NUMBERS —				REMARKS, RELATIVE LENGTHS, ETC. †
	1*	2	3	4	
Hind Leg					
Femur	0·585	0·628	0·521	0·557	
Tibia	0·771	0·830	0·714	0·730	= $\frac{1}{2}$ length leg
Tarsus, seg 1	0·357	0·393	0·314	0·335	
Tarsus, segs 2—5	0·543	0·600	0·500	0·528	= $\frac{1}{4}$ length leg.
Total length	2·24	2·45	2·05	2·15	(not including coxa and trochanter)
Genitalia.					
Sup clasper, seg 1	0·200	0·219	0·198	0·190	=2·26—2·43 $\times$ seg 2, 1·14—1·22 $\times$ intermed append, 1·10—1·25 $\times$ inf clasper
Sup clasper, seg 2	0·084	0·090	0·084	0·079	
Intermed append	0·174	0·180	0·165	0·156	=inf. clasper
Intromit organ	0·123	0·144	0·135	0·126	
Inferior clasper	0·171	0·195	0·168	0·156	
Subgen lamellæ	0·150	0·165	0·150	0·135	=0·84—0·89 $\times$ inf clasper
Genital filament	0·057	0·054	0·036	0·000	(length protruded).
Pompetta, length	0·090	0·099	0·087	0·084	
Spines on seg 2	0·081	0·090	0·087	0·078	=length seg 2

\* Type male

† Data from 8 specimens

The total *length* of the mounted specimens varied from 1·8 to 2·1 mm. This length averaged about 0·89 times that of the hind leg and 1·42 that of the wing.

The *buccal cavity* (Plate LVII, fig 4) usually shows no sign of a pigmented area, but in some specimens a very faint and small trace may be detected. The teeth are small and poorly developed. They are arranged in a single curved row.

The *pharynx* (Plate LVII, fig 3) is much narrower than in the female and the teeth only show as short ridges with serrated edges.

The *palps* (Plate LVII, fig 2) have a formula of 1, 2, 3, 4, 5. The relative lengths of the segments averaged 2·7, 6·1, 8·7, 10, 22·0. Newstead's spines are in the basal third of the 3rd segment and number about 8 or 10. The combined lengths of segments 1 and 2 is about equal to that of segment 3, while the 4th segment forms about  $\frac{1}{5}$ th of the total length of the palp. The ratio palp over epipharynx is about 3·9.





In *P. simillimus* the end of the IIIrd antennal segment projects beyond the tip of the proboscis, this segment is also longer than in *P. barraudi*. The inferior clasper is stated by Newstead to project 'beyond the submedian process to a distance a little less than one-fourth its length' in *P. simillimus*. It does not do so in *P. barraudi*.

## REFERENCES

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| NEWSTEAD, R (1914)             | <i>Bull Entom Res</i> , V, 2, pp 180—182           |
| SINTON, J A (1927)             | <i>Ind Jour Med Res</i> , XV, 1, pp 29-31          |

Barhi	34	..				34	.	34	.	34	..	16
Barakatha	50	1				49				50		
Chopra	67					67			.	67		
Town Balasore	31	5	11	2	3	20	.		5	26	..	
Hospital "	14	2	3	2		11			2	12		
Police "	80	13	21	4	9	59			13	67		
Chowkidars "	107	26	5	3	23	102			26	81		
Schools "												
Industrial	52	9			9	52			9	43		
Zilla	75	10			10	75			10	65	..	
Mission	31	3	4		3	27			3	28		
Orphanage	77	8			8	77			8	69		
Town Gaya	24	3	10	1	2	14			3	21	..	
Police "	100	11	1	.	11	99			11	89	..	
Total	2,321	328	285	50	278	2,036	14	188	322	1,821	8	

# EXPLANATION OF PLATE LVI

Camera-lucida drawings of parts of *P. baraudi* (♀)

- Fig 1 Wing
- „ 2 Palp, showing the 'spines of Newstead' (N)
- „ 3 Segments XII to XVI of the antenna, showing the geniculate spines
- „ 4 Segments III and IV of the antenna
- „ 5 Spermatheca
- „ 6 Furca
- „ 7 Post-genital plate and spines
- „ 8 Dorsal plate of pharynx
- „ 9 Buccal cavity, showing pigmented area and teeth

area such a result was 2 and 22 per cent respectively. Reading the results of the day and night examinations in all the areas investigated they show a 7 per cent positive result where the examinations were conducted during the day time and a 15 per cent positive result during the night time.

It is clear, therefore, that a night examination of the blood is essential to arrive at a true situation where filariasis is to be diagnosed by the presence of the parasite in the peripheral blood.

(v) Incidence of filariasis according to age and sex

The difficulty in examining the female and the juvenile population of the agricultural section in the areas of Bihar was more pronounced than in the areas of Orissa. On this account the investigation in this section of the population was smoother in the Balasore district area. The results are given in the form of a table (Table IV).

The table shows that 17 per cent of males and 11 per cent of females are infected by filaria in the Balasore district.

The juvenile age has been taken between the group of 0 to 20 years. In the case of juvenile males, 5, 22, 14 and 14 per cent show the presence of filariasis in the Gaya, Patna, Purnea and Balasore districts respectively, and in the case of females, 12 per cent in the Balasore district only.

## B Morphology of *MICROFILARIA BANCROFTI*

### (i) Human Phase

The morphological details of the microfilaria differ in no way from what has been studied before (Korke, 1928).

Deviations from the type species of *bancrofti* have been observed in some of the areas of the Balasore district and the observations form a part of a separate communication.

### (ii) Mosquito Phase

Developmental stages of *F. bancrofti* in mosquito

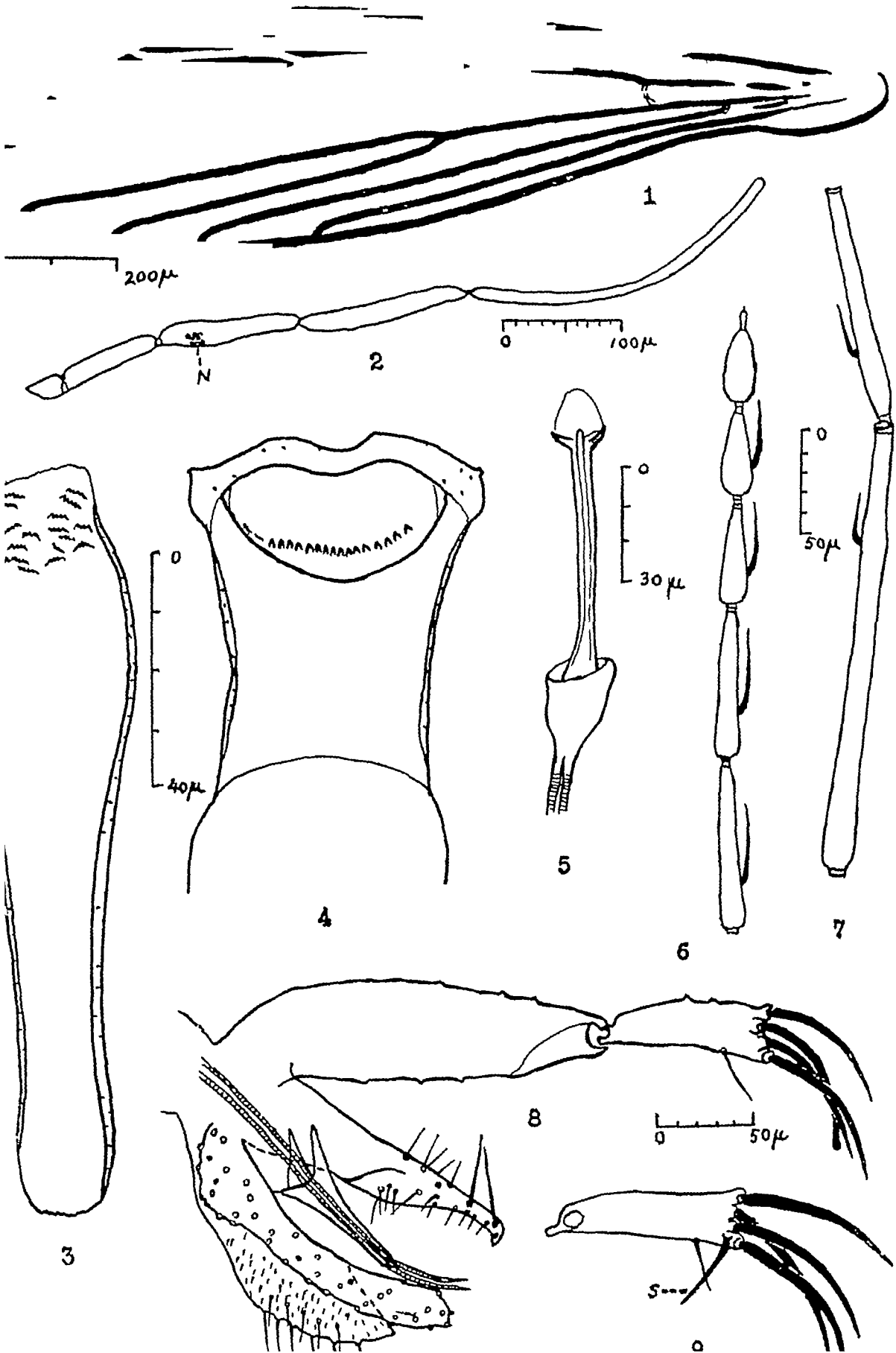
Expt 1, 11 mosquitoes collected from the General Hospital, Balasore, on 13th June 1928 and dissected after one day, result, 3 positive or showing cylindrical bodies possessing rudimentary alimentary canal with a refractile anal pore. Some bodies were in degenerated forms. Temperature 83.5 dry and 79.8 wet.

Expt 2, 3 mosquitoes collected from the above area on the same date, dissected 3 days after, positive 2, result similar to No. 1. Temperature similar to above.

Expt 3, 4 mosquitoes collected from the above area on the same date and dissected 4 days after, result negative.

Expts 4 and 5, 5 mosquitoes collected from the Police Station, Balasore, on 15th June 1928 and dissected after 2nd and 3rd day with negative result.

Expt 6, 23 mosquitoes collected from the above area on 18th June 1928 and were dissected after 2 days, 6 were found to be positive. One mosquito showing proboscis infection and the rest containing cylindrical and sausage-shaped bodies in various stages of development. Temperature 79.6 dry and 77.8 wet.



Expts 7, 8, 9 and 10, 47 mosquitoes were collected from hospital, Bhadrak (30th June 1928), Police Station, Soro (13th July 1928), Police Hospital, Balasore (16th July 1928) and Police Station, Jalleswar (17th July 1928), and dissected at the interval of a few hours to a day, showed negative results

Expts 11, 12, 13 and 15, 89 mosquitoes were collected from Superintendent of Police's quarters, Balasore (19th July 1928), Sadr Hospital, Balasore (19th July 1928), from Outpost, Balasore (21st July 1928), and Police Lines, Balasore (22nd July 1928), and were dissected at the interval of a few hours to one day, result 10 were found to be infected showing forms of human phase, intermediate forms to filari-forms infecting proboscis Temperature ranged from 79.2 to 84.1 dry and from 78 to 80 wet

Expts 14, 16, 17, 18, 19 and 21, 85 mosquitoes were dissected from Balasore area between the dates 20th July 1928 and 23rd July 1928 and were dissected at the interval of few hours to 3 days all showing negative results

Expts 20, 22, 23 and 24, 82 mosquitoes were dissected from Balasore, Bhadrak and Basudebpur between the dates 23rd July 1928 and 29th July 1928 and were dissected at the interval of 1 to 6 days, result developmental forms showing human phase to proboscis infection were found in 8 Temperature varied between 80.2 to 83.1 dry and 77 to 81 wet

Total result 349 mosquitoes were dissected between the dates 13th June 1928 and 29th July 1928 in the Balasore area out of which 29 showed developmental stages of *F bancrofti*, 3 mosquitoes showing proboscis infection Percentage of infection 8.3

The preponderance of the species of mosquitoes in the areas investigated (sub-genera not shown)

<i>Culex fatigans</i>	Arwal, Bhalua, Gaya, Manair, Paliganj, Barhi, Balasore, Bhadrak, Chandipur, Jalleswar, Kishanganj, Chopra
<i>C. vishnu</i>	Bhalua, Balasore, Bhadrak, Jalleswar
<i>Temorhynchus annuliferus</i>	Balasore, Bhadrak, Soro, Basudebpur
<i>T. uniformis</i>	Balasore, Jalleswar, Soro, Basudebpur
<i>C. bitemorhynchus</i>	Bhalua, Bhadrak, Jalleswar
<i>Anopheles culicifacies</i>	Arwal, Bhalua, Paliganj
<i>A. subpictus</i>	Bhalua, Barhi
<i>C. epidesmus</i>	Jalleswar, Basudebpur
<i>Lutzia fuscana</i>	Bhalua, Jalleswar
<i>Aedes albopictus</i>	Paliganj
<i>A. vittatus</i>	Bhadrak
<i>A. macropterus</i>	Balasore
<i>Anopheles fuliginosus</i>	Chopra
<i>Armigeres obturbans</i>	Bhadrak
<i>C. pullus</i>	.. Bhalua
<i>C. whitmorei</i>	, Jalleswar.



district But density in a purely agricultural area (as opposed to the industrial area) is largely dependent upon the conditions of agriculture and favourable conditions of agriculture in an alluvial tract largely depend upon the soil and moisture These are all associated factors, one cause recoiling on the other

In the submontane or plateau areas so far investigated and where the conditions of normal agriculture are not satisfied, the results have proved negative for filariasis

## 2 *The species of mosquito responsible for the transmission of the disease*

The life-cycle of *bancrofti* has partly been described in *Culex fatigans* in the Gaya district (Korke, 1928) *C fatigans* appears to be present in almost all the areas investigated In addition to the above species, *C vishnu*, *T annuliferus* and *T uniformis* are abundantly present in the areas, which have contributed to a high degree of infection in filaria, viz, the Balasore district area Deviations from the type species of *bancrofti* (not published in this paper) are found to be present in the above area It is highly probable, that the infection is kept up not only by the *fatigans* but by the above species of mosquitoes as well It is also probable that the adaptation of *bancrofti* in the new species of mosquito has resulted in deviated forms of *bancrofti*, but the information on this point is not complete

## 3 *The clinical signs and symptoms*

The male population has mainly been investigated A notable feature in the incidence of filariasis is that a large section of population (85 per cent), which show microfilaria in the peripheral blood do not exhibit any clinical signs and symptoms (Table I-A)

In the agricultural section of the population of the Balasore area 18 per cent of positive cases do not exhibit any signs and symptoms (total cases 943, positive 169) The total cases include a number which has been investigated during the day and hence the percentage shown is virtually the minimum

Similarly in the areas above the sea-level, the percentage is 9 (total cases 254, positive 24) In the Sone canal area the percentage is 12 (total cases 255, positive 30)

The clinical signs refer mostly to glands, tunica vaginalis, testicle, and lower extremity In the areas at the sea-level the affections of the lower extremity stand first, tunica vaginalis stands second and glands stand last In the areas above the sea-level affections of tunica vaginalis stand first, glands second and testes the last

In a large percentage of cases parasites have been detected in the peripheral blood where the affections relate to the tunica vaginalis and the inguinal glands, but this is not so where the affections relate to the lower extremity

## 4 *The time for blood examination (to get the optimum result) where the infection is by bancrofti*

The periodicity of *bancrofti* is a well marked feature and I have shown that during the phase of 24 hours the parasites practically disappear from the



The researches at Liverpool (Stephens, *et al*, 1917—1921) and at Dagshai (Rennie, Acton, Curjel and Dewey, 1921, Acton, Curjel and Dewey, 1921) seem to be the only instances in which systematic blood examinations were carried out over long periods to test the anti-relapse powers of these drugs in benign tertian malaria. Although a large amount of research was carried out in these places, it was mainly concerned with the more popular alkaloid, quinine, and very little has been done to test the other three crystallizable alkaloids along the same lines.

The results recorded in this paper have been obtained during 5 years of work on the anti-relapse treatment of malaria, and during that time over 1,300 cases of benign tertian malaria have been treated with the different cinchona alkaloids.

#### DETAILS OF METHODS, CONTROLS, ETC., USED IN THE TESTS

Sinton (1926) suggested certain steps, which he considered essential for the scientific testing of the efficacy of any treatment for the production of a permanent cure in malaria. In the research here recorded, these methods have been followed with care, except for the few instances mentioned in the text.

The British patients were soldiers treated in the hills at Kasauli, under conditions which precluded any chance of reinfection, and were of the same type of population as that described in detail in previous articles of this series (Sinton *et al*, 1926—1928).

The Indian patients were adult male prisoners in the Lahore Central Jail, but were unfortunately treated under conditions which did not exclude the chance of reinfection, during either the period of treatment or of observation after treatment (Sinton, 1923).

In the experiments British patients were separated from Indians to exclude any fallacies due to immunity, either natural or acquired, which might affect the relapse rate in the two races.

Fresh infections have, as far as possible, been dealt with separately from the chronic relapsing ones, for it seemed probable that the relapse rate differed markedly in these two stages of the disease.

Relapses, as heretofore, were diagnosed by the finding of parasites in the peripheral blood, when examined systematically each week for eight weeks after the cessation of all treatment. The examinations were made by the thick-film method.

In all tests at least two treatments, of which one was a quinine treatment, were carried on at the same time, so that the results of one treatment might act as a control on the other. In several instances, three or even four forms of treatment were conducted during the same period, the alternative case method being used (Sinton, 1926). In this way one form of treatment tested on a large number of patients would act as control for several shorter series running concurrently with it. It is not proposed to repeat under each treatment the

11 8 3 per cent of mosquitoes, species not certain, but mostly *culex*, showed developmental stages of *bancrofti* in the Balasore area

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- BENGAL DISTRICT GAZETTEERS Balasore (1907), Gaya (1919), Hazaribagh (1917),  
Patna (1924) and Purnea (1911)

From this table it will be seen that of 29 patients treated with quinine, only one relapsed. This was a patient who had a mixed infection with *P. vivax* and *P. falciparum*. The relapse rate after quinine was therefore only about 3.5 per cent. The patients treated with the other alkaloids were too few in number to allow any conclusions to be drawn as to the relative values of the different drugs.

In addition to the cases recorded in Table I, the records of another fifteen patients is available. These patients had a history of previous malaria, but had had no clinical symptoms for periods varying from 8 to 13 months previous to the attack for which they were treated. Whether these were fresh infections or relapses after a long interval, could not be determined. The results of the treatment of these patients is shown in Table I(a).

TABLE I(a)

Treatment	Grams of drug $\times$ days of treatment	Total drug — days of treatment	Number of patients	Number of relapses
QA	$30 \times 7, T \times 7, 20 \times 7$	350 — 21	3	2 (66.6 per cent)
QA	$30 \times 14, 20 \times 7$	560 — 21	8	4 (50.0 per cent)
QA	$30 \times 7, 20 \times 24$	690 — 31	3	1 (33.3 per cent)
QA	$30 \times 7, T \times 7, 30 \times 7, 10 \times 35$	770 — 56	1	0
		TOTAL	15	7 (46.6 per cent)

The relapse rate in these cases is much higher than those in Table I. It may be that these patients had either relapsing infections or represent some of those patients whom James (1924) considered to be abnormally susceptible to the disease and hence they had contracted new infections shortly after being cured of the previous ones.

A total of thirty-eight British patients known to be suffering from fresh infections with *P. vivax* were treated with the different alkaloids and showed a relapse rate of about 8 per cent. While the rate among the total of 53 patients recorded in this section is about 19 per cent.

#### (b) Indian Patients

These patients were adult male prisoners in the Lahore Central Jail and, as most Indians have a history of 'fever' at some period of their lives, it was impossible to ascertain how many of these suffered from fresh infections and how many had relapsing ones. As far as could be discovered from their

The integument of the body was very dark brown, almost black, except for the sides of the thorax, the coxæ and the trochanters, which were brownish, or greyish yellow. The antennæ, proboscis and palps were dark grey, except for the last two segments of the latter, which were more silvery.

The clypeus had a large tuft of hair-like scales on the dorsum directed in a forward and upward direction. The wings, palps and antennæ were covered with dark grey hairs and scales with silvery reflections. The wings showed a golden iridescence with a slight bluish tinge in parts.

The legs had a silvery sheen, but looked almost black in some lights.

*Appearances in Stained and Mounted Specimens.*

The measurements of the Type Female and of three other specimens are given in Table I, while the ratios, etc., of nine specimens are also recorded.

TABLE I.  
*Phlebotomus Barraudi*, N. SP. (♀).

Structure	LENGTH IN MM OF SPECIMENS NUMBERS —				REMARKS, RELATIVE LENGTHS, ETC. †
	*1	2	3	4	
Body					
Clypeus and head	0.400	0.357	0.343	0.343	
Thorax	0.643	0.585	0.570	0.543	
Abdomen proper	1.330	1.285	1.257	1.285	
Total length	2.40	2.23	2.20	2.03	=1.30—1.42 × wing, 0.83—0.97 × leg,
Labium	0.228	0.214	0.214	0.214	reaches beyond antenna seg. III
Epipharynx	0.195	0.195	0.195	0.195	
Antenna					
Segment III	0.207	0.193	0.178	0.182	III=IV+V III=IV=V
Segment IV	0.100	0.100	0.093	0.093	IV+V+VI<XII-XVI
Segment V	0.100	0.100	0.093	0.093	Antennal formula. $\frac{2}{\text{III-XV}}$
Segment VI	0.100	0.100	0.093	0.093	
Segments XII-XVI	0.343	0.314	0.300	0.307	=1.63—1.70 × IIIrd
Total length	1.514	1.385	1.300	1.328	=7.2—7.3 × IIIrd, 4.2—4.4 × XII-XVI

\* Type female

† Data from 9 specimens.

chronic cases are included, and (c) the chances of reinfection were not excluded. Even in spite of these facts, the rate is lower than that found in the treatment of chronic relapsing cases.

(c) *Patients Observed Clinically*

The patients in the preceding series were all observed by weekly blood examinations for 8 weeks after the end of treatment. A number of Indian patients were also observed in which this method was not possible. These patients, as in the other Indian series, probably contained both fresh and chronic cases. They were drawn from the jail staff and were observed by blood examination whenever any clinical symptoms suggestive of relapse were noted, and also at the end of eight weeks after the cessation of treatment. The results are shown in Table III.

TABLE III

Series	Grains of drug × days of treatment	Total drug — days of treatment.	Number of patients	NUMBER OF RELAPSES			PERCENTAGE OF RELAPSES		
				BT	MT	Total	BT	MT	Total
13 QA	20 × 1, 30 × 3	110 — 4	32	6	0	6	18·7	0	18·7
14 QA	30 × 7	210 — 7	12	0	2	2	0	16·6	16·6
		Total	44	6	2	8	13·6	4·5	18·1

The results in these series are better than those in Table II, which may be partly accounted for by the fact that relapse is not so likely to be detected in 8 weeks of observation by clinical methods as it is by blood examination.

THE RELAPSE RATE IN FRESH INFECTIONS

The opinion formed by many workers during the Great War was that fresh infections of *P. vivax* were more easily cured than the chronic relapsing ones. The results of the treatment of benign tertian malaria induced by the inoculation of blood for the cure of mental diseases goes to show that such infections are cured in the great majority of cases by therapeutic doses of quinine given for less than one week. However, the results reported in the treatment of therapeutic malaria conveyed by mosquito bites have not been so satisfactory. Unfortunately,

The *buccal cavity* (Plate LVI, fig 9) has a well-marked toadstool-like pigmented area, the stalk of which is inclined to be ragged or fenestrated at the end. The armature has about 40 well-developed teeth arranged in a curved line.

The *pharynx* (Plate LVI, fig 8) is not much dilated at its posterior portion and is lined at this end by numerous teeth with very long, thin and pointed ends, which form a dense brush posteriorly. The arrangement rather resembles that in *P. malabaricus*. The length of the pharynx is about 2.6 times its breadth.

The *palps* (Plate LVI, fig 2) have a formula of 1, 2, 3, 4, 5, and the relative lengths of the segments in order averaged 2.9, 6.3, 8.8, 10.0, 22.4. The combined lengths of segments 1 and 2 equal that of 3, while the 4th segment forms about 1/5th of the total length of the palp. The average of the ratio palp over epipharynx is about 3.5, but in some specimens the terminal segment of the palp is relatively short, with the result that the ratio is smaller than the average. The 'spines of Newstead' are situated on the basal third of the 3rd segment and number about 25. The total length of the palp is about half that of the antenna.

The *antennæ* (Plate LVI, figs 3 and 4) have a total length averaging about 7.27 times that of its IIIrd segment, 4.33 times that of the combined lengths of segments XII—XVI and 0.83 times that of the wing length. The IIIrd segment is long but does not extend beyond the end of the proboscis. It is shorter than the same segment in the male, and equals the combined lengths of segments IV and V. The combined lengths of segments XII—XVI averaged about 1.67 times the length of segment III. The antennal formula is 2 over III—XV, and the spines are of medium length, those on the terminal segments almost reaching the next articulation.

The *wing* (Plate LVI, fig 1) is about 3.8 times as long as broad and its length is about 0.68 times that of the hind leg. The average of the different ratios of the wing venation were as follows —

$\alpha$  over  $\beta$ , 0.93,  $\beta$  over  $\gamma$ , 1.08,  $\alpha$  over  $\gamma$ , 1.05,  $\delta$  over  $\alpha$ , 0.44,  $\alpha$  over  $\epsilon$ , 0.68;  $\theta$  over  $\epsilon$ , 2.01,  $\beta$  over  $\epsilon$ , 0.72,  $\alpha + \beta$  over  $\theta$ , 0.70 and wing length over  $\theta$ , 2.0. The wing breadth is almost equal to  $\beta$ .

The length of the *hind leg* is about 3 times the length of its tibia and  $6\frac{1}{2}$  times that of its 1st tarsal segment.

The *genitalia* (Plate LVI, figs 5, 6 and 7) have a smooth spermatheca and the post-genital ridge has usually two spines, but three have been found in some specimens.

#### Differential Diagnosis

The absence of erect hairs on the dorsum of the abdomen and the smooth spermathecae differentiate this species from all the members of the 'erect-haired' group.

The morphology of the buccal cavity, the wing venation and other points differentiate *P. barraudi* immediately from *P. himalayensis*, *P. scylanicus*,

*(b) Effects of treatment in curing infections following mosquito bite*

Yorke and Macfie (1924) found that after a treatment of 30 grains of quinine daily for 3 consecutive days, of 31 patients who survived, four or 13 per cent relapsed. In a later paper Yorke (1925) reports that of 37 patients infected by this method, 57 per cent relapsed after a similar course of treatment.

Rudolf (1927) has collected details of 31 patients of whom 19 or 61 per cent relapsed after various treatments. Of 3 patients treated by this author with 200 grains of quinine in 17 days ( $20 \times 3, 10 \times 14$ ), two or 66.6 per cent relapsed.

James (1926), with a treatment of 30 grains of quinine daily for 5 days, records a relapse rate of about 25 per cent. Nicol (1927) tried two forms of treatment, one of 30 grains daily for 5 days and one of 15 grains daily for 10 days, the total amount of quinine given being 150 grains in both cases. He states that 'in about 50 per cent of cases a relapse of the malarial attack occurs'.

It must be remembered, in considering these relapse rates, that patients who die after artificial malarial infections are not included and Nicol (1927) states that 6 per cent of all patients treated died. No indication is given, however, as to how many of these died from the induced disease and how many from other causes. Deaths due to malaria should be considered as failures in treatment and included in the relapse rates.

These results go to show that in therapeutic malaria, at least, infections conveyed by the mosquito are more liable to relapse than those transmitted by the direct inoculation of blood. The results of the treatment of patients infected by the latter method would, therefore, appear not to be comparable with the results obtained in infections conveyed by the natural method. James (1924) thinks that the virulence of his strain of *P. vivax* has been enhanced by repeated passage through the mosquito.

The relapse rate in the 38 fresh infections recorded in Table I, in which long courses of the cinchona alkaloids were given, was only about 8 per cent. When short courses of these alkaloids were used, as with the 154 patients recorded in Table II, the rate was 36 per cent. If relapses due to *P. vivax* are considered only, the rate in these series is about 30 per cent, which is low when one considers that these patients included both fresh and chronic infections and were treated under conditions which did not preclude reinfection.

*(c) Discussion of results*

If the series in Tables I(a) and III are included, the total number of patients observed was 251 of whom about 24 per cent relapsed. These results are very similar to those reported by James (1926) in the treatment of malaria following mosquito bite. The facts that some chronic cases are included in our series, and that in some instances reinfection was probable as a cause of 'relapses,' are possibly compensated for by the fact that some of the series received longer treatment and larger amounts of quinine than James's cases.

The patients recorded in Tables II and III are probably a fair sample of the average type of adult male population found infected with *P. vivax* in India. The

TABLE II—(contd)

Structure	LENGTH IN MM OF SPECIMENS NUMBERS —				REMARKS, RELATIVE LENGTHS, ETC †
	1*	2	3	4	
Antenna					
Segment III	0.221	0.228	0.207	0.200	III=IV + V IV=V=VI
Segment IV	0.110	0.114	0.103	0.100	IV + V + VI > XII—XVI
Segment V	0.110	0.114	0.103	0.100	Antennal formula $\frac{1}{\text{III—XV}}$
Segment VI	0.110	0.114	0.103	0.100	
Segments XII—XVI	0.307	0.314	0.286	0.286	=1.37—1.49 × IIIrd.
Total length	1.457	1.485	1.386	1.370	=6.56—6.90 × IIIrd, 4.71—4.95 × XII—XVI.
Palp					
Segment 1	0.036	0.036	0.033	0.033	Formula 1,2,3,4,5,
Segment 2	0.078	0.084	0.075	0.072	Relative lengths 2.7, 6.1, 8.7, 10, 22.0
Segment 3	0.111	0.120	0.108	0.102	=½ length palp $\frac{P}{E} = 3.85—4.14$
Segment 4	0.129	0.138	0.123	0.114	
Segment 5	0.288	0.330	0.258	0.255	=0.42—0.45 × antenna, 0.42—0.46 × wing
Total length	0.642	0.708	0.597	0.576	
Wing					
Length	1.470	1.530	1.257	1.347	=4.18—4.48 × breadth, 0.61—0.65 × leg
Breadth	0.343	0.357	0.293	0.320	$\frac{\alpha}{\beta} = 0.81—1.0$ $\frac{\beta}{\gamma} = 0.95—1.0$
$\alpha$	0.250	0.257	0.207	0.228	
$\beta$	0.278	0.285	0.214	0.235	$\frac{\alpha}{\gamma} = 0.86—0.97$ $\frac{\delta}{\alpha} = 0.40—0.48$
$\gamma$	0.285	0.285	0.214	0.243	$\frac{\alpha}{\epsilon} = 0.69—0.71$ $\frac{\beta}{\epsilon} = 0.70—0.76$
$\delta$	0.121	0.114	0.086	0.100	
$\epsilon$	0.343	0.371	0.293	0.321	$\frac{\theta}{\epsilon} = 2.0—2.14$ $\frac{\alpha + \beta}{\theta} = 0.68—0.70.$
$\theta$	0.743	0.771	0.607	0.657	
$\pi$	0.071	0.085	0.071	0.085	$\frac{\text{Wing length}}{\theta} = 1.99—2.07$

\* Type male.

† Data from 8 specimens



The average relapse rate for Series 15 to 19 was 67·8 per cent

TABLE IV

Series	Grains of drug × days of treatment (a)	Total drug — days of treatment	Total number of patients	Number lost sight of	Number not relapsing	Number of relapses	PERCENTAGE OF RELAPSES			
							Observed (b)	Possible maximum (c)	Observed minimum (d)	Average (e)
15 Q	24 × 10	240 — 10	103	7	21	75	78·1	79·6	72·8	76·8
16 Q	20 × 1, 30 × 6, T × 7, 20 × 7	340 — 21	63	1	11	51	82·2	82·5	80·9	82·3
17 Q	30 × 14, 20 × 7	560 — 21	91	19	22	50	69·4	75·8	55·0	66·5
18 Q	30 × 7, T × 7, 30 × 7, 10 × 35	770 — 56	51	2	16	33	67·3	68·6	64·7	66·8
19 Q	30 × 14, 10 × 42	840 — 56	114	9	49	56	53·0	57·0	49·1	53·2
		TOTAL	442	38	119	265	69·0	71·8	62·8	67·8

(a) 'T' is an iron and arsenic tonic.

(b) The 'observed relapse rate' is obtained by excluding the cases 'lost sight of' from the total number of cases

(c) The 'possible maximum rate' is obtained by considering all cases 'lost sight of' as possible relapses

(d) The 'observed minimum rate' is the percentage of relapses observed among the total number of patients treated

(e) The 'average relapse rate' is calculated from all the figures on which the three previous rates were estimated

(u) *Quinine and Alkali*—The details of this method of treatment have been described in full by Sinton (1923a, 1926a), and the reasons for its use discussed

The duration of treatment lasted from 21 to 56 days during which periods from 340 to 840 grains of quinine sulphate was given in solution by the mouth

The *antennæ* (Plate LVII, figs 6 and 7) have a formula of 1 over III—XV, with spines of moderate length. The IIIrd segment is longer than in the female but does not reach as far as the end of the proboscis. The length of this segment is equal to the combined lengths of segments IV and V. Segments XII—XVI are about 1.39 times as long as the IIIrd. The total length of the structure averages about 6.7 times the length of the IIIrd segment, 4.81 times segments XII—XVI and is about equal to the length of the wing.

The *wing* (Plate LVII, fig. 1) is smaller and relatively narrower than in the female, the ratio length over breadth averaging 4.28. The averages of the different ratios of the wing venation was as follows —

$\alpha$  over  $\beta$ , 0.93,  $\beta$  over  $\gamma$ , 0.98,  $\alpha$  over  $\gamma$ , 0.91,  $\delta$  over  $\alpha$ , 0.42,  $\alpha$  over  $\epsilon$ , 0.70,  $\theta$  over  $\epsilon$ , 2.08,  $\beta$  over  $\epsilon$ , 0.74,  $\alpha + \delta$  over  $\theta$ , 0.69 and wing length over  $\theta$ , 2.04. The wing breadth almost equals  $\epsilon$ .

The length of the hind leg is about 4 times that of tarsal segments 2—5, and three times the tibia. The femur slightly longer than the tarsal segments 2—5.

The *genitalia* (Plate LVII, figs 5, 8 and 9) are very like those of *P. minutus*, but larger. The proximal segment of the superior clasper averaged 2.34 times the length of the distal segment, 1.2 times the intermediate appendage and 1.16 times the inferior clasper. The inferior clasper and the intermediate appendage are approximately of the same length. The subgenital lamellæ is about 0.86 times the length of the inferior clasper. The genital filaments are usually protruded for a short distance and the pompetta lies in the 6th or 7th abdominal segments.

The longest spines on the distal segments of the superior clasper are as long as the segments which carry them.

One specimen showed an abnormal 6th spine on the distal segment of the superior clasper (Plate LVII, fig. 9).

### Differential diagnosis

Among the recumbent haired group the species has to be separated from *P. minutus*, *P. babu*, *P. shortti*, *P. montanus* and *P. squamipleuris* in India. The poor development of the buccal and pharyngeal armatures in all these species make this a matter of some difficulty.

The wing venation in the first three species is very different from that of *P. barraudi*. The absence of geniculate spines on the IIIrd segment of the antenna and the presence of Newstead's spines on the 2nd palpal segment help to differentiate *P. squamipleuris*, which also shows pleural scales which are absent in *P. barraudi*. *P. squamipleuris* has a very flask shaped pharynx.

The species shows a resemblance to *P. montanus*, but that species has a better developed pigmented area and more teeth (cf. Sinton, 1927, Plate VIII, fig. 11). The spines on the superior clasper are arranged two terminal and two subterminal in *P. montanus*, not almost terminal as in *P. barraudi*.

## (b) Quinidine Treatment

(i) *Quinidine only*—Quinidine sulphate was given by the mouth in these cases. The solutions used are shown in Appendix A.

Series 24 QD, 25 QD, 28 QDA and 29 QDA were treated at the same time as Series 16 Q, 17 Q, 20 QA and 21 QA, while 26 QD was carried out with 18 Q, 22 QA, 34 CF and 35 CFA, and 27 QD with 19 Q and 23 QA.

As shown in Table VI a total of 120 patients were treated, followed by an average relapse rate of 81.5 per cent. The 'after histories' of six of the 8 cases 'lost sight of' in Series 26 QD were traced. Of these two had relapsed and four had shown no clinical symptoms of malaria up to the end of 6 months, nor had three of the four cases in Series 25 QD.

TABLE VI

Series	Grains of drug $\times$ days of treatment	Total drug — days of treatment	Total number of patients	Number lost sight of	Number not relapsing	Number of relapses	PERCENTAGE OF RELAPSES			
							Observed	Possible maximum	Observed minimum	Average
24 QD	20 $\times$ 7, 20 $\times$ 7, 20 $\times$ 7	280 — 21	50	1	6	43	87.7	88.0	86.0	87.2
25 QD	20 $\times$ 21	420 — 21	40	4	6	30	83.3	85.0	75.0	81.0
26 QD	21 $\times$ 7, 14 $\times$ 24	483 — 21	14	8	0	6	100.0	100.0	43.0	76.4
27 QD	20 $\times$ 28	560 — 28	16	0	5	11	68.7	68.7	68.7	68.7
		TOTAL	120	13	17	90	75.0	85.8	84.1	81.5

(ii) *Quinidine and Alkali*—These patients were treated with quinidine sulphate in combination with the alkali treatment of Sinton. The controls for these series are shown under 'quinidine only'.

The results of the treatment of 88 patients is shown in Table VII. The average relapse rate was 84.8 per cent. One of the relapses in 28 QDA was due to *P. falciparum*.



## (d) Cinchonidine Treatment

These patients were given cinchonidine sulphate in solution. Of the 19 cases 'lost sight of' in Series 31 CD, thirteen gave no clinical history of relapse up to 6 months after completing observation, while four had relapsed. The remaining two were not traced.

Series 31 CD was controlled by Series 16 Q and 17 Q, while Series 32 CD was controlled by Series 19 Q.

The average relapse rate as shown in Table IX was 68.7 per cent in 107 cases.

TABLE IX

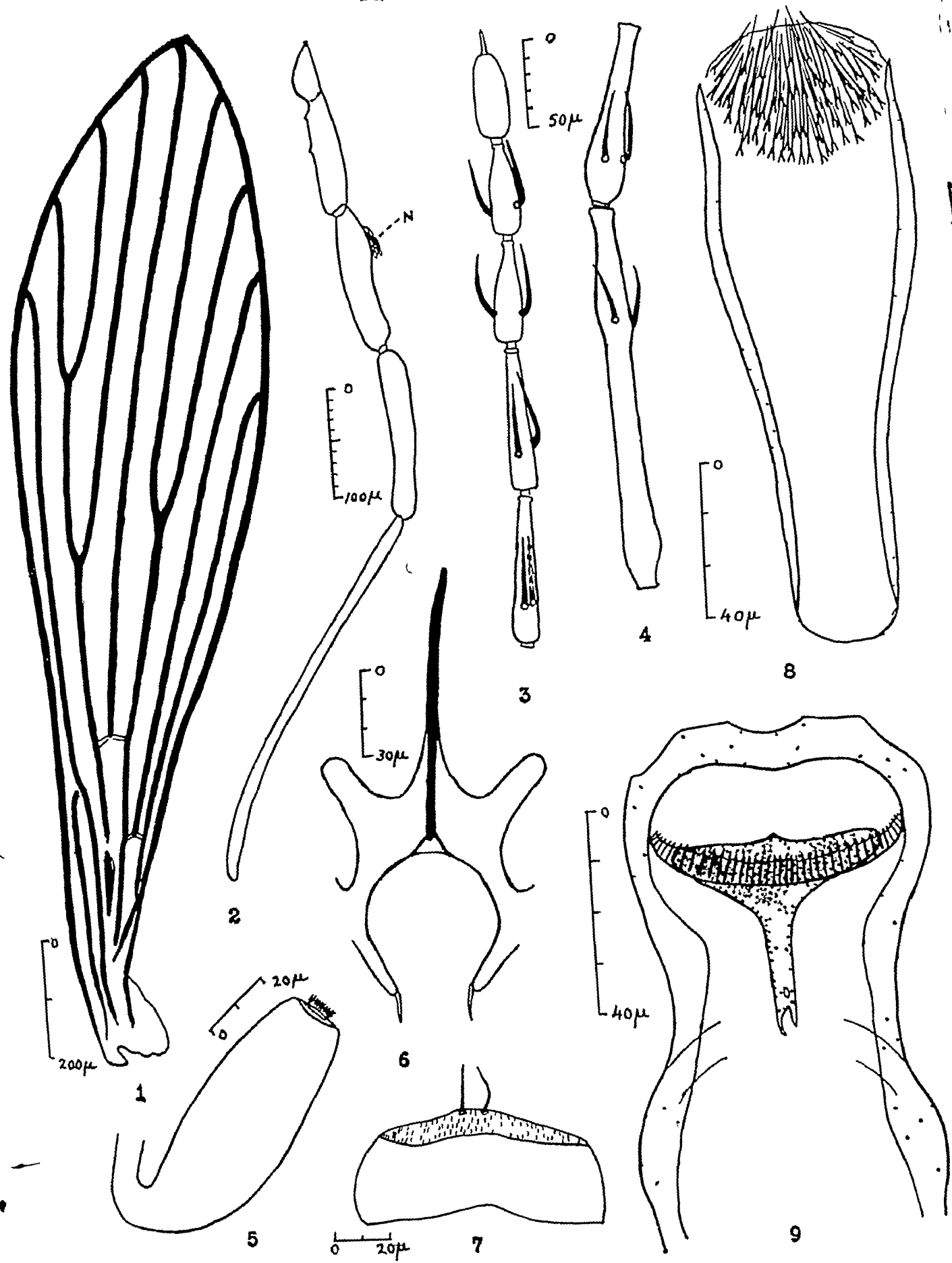
Series	Grains of drug $\times$ days of treatment	Total drug — days of treatment	Total number of patients	Number lost sight of	Number not relapsing	Number of relapses	PERCENTAGE OF RELAPSES			
							Observed	Possible maximum	Observed minimum	Average
31 CD	20 $\times$ 21	420 — 21	30	19	1	10	91.0	96.6	33.3	69.0
32 CD	20 $\times$ 28	560 — 28	77	5	22	50	69.4	71.4	64.9	68.5
		TOTAL	107	24	23	60	72.3	78.5	56.0	68.7

## (e) Cinchona Febrifuge Treatment

Cinchona febrifuge or Kinetum was originally a mixture of the total alkaloids obtained from the bark of *Cinchona succubra*, but in more recent years it has contained some of residual alkaloids of the bark of *C. ledgeriana* after a certain amount of the quinine has been extracted. It, therefore, contains all the four crystallizable alkaloids previously mentioned and also the amorphous alkaloid, quinidine. As might be expected, the composition of the drug varies very considerably according to the proportions of the different alkaloids in the samples of bark from which it is manufactured. Series 33 CF was treated with 'Malarene,' which is a cinchona febrifuge manufactured by the Madras Government Cinchona Factory. In this preparation the makers have tried to place a drug of standardized composition on the market.

Series 33 CF was controlled by Series 19 Q and 25 QA. The controls of Series 34 CF and 35 CFA were Series 18 Q and 22 QA.

Of the 12 cases in Series 34 CF, who were 'lost sight of,' eight gave no history of malaria and three had relapsed clinically during the six months after



on researches of this nature that the selection of quinine, as the alkaloid of choice in the treatment of malaria, was founded

In Table XI are shown the results of our research on the treatment of over 1,100 cases of chronic relapsing benign tertian malaria with the four crystallizable cinchona alkaloids and with cinchona febrifuge

#### (a) *Quinine Treatment*

Quinine treatment was used in 667 cases with an average relapse rate of 68.0 per cent (maximum 86.2, minimum 48.3), which is 3.2 per cent lower than the average for all the cases treated with the cinchona alkaloids

Rennie, Acton, Curjel and Dewey (1921) treated 663 British soldiers suffering from chronic benign tertian malaria with a relapse rate of 67.6 per cent. They concluded that no form of quinine treatment would cure more than 25 to 30 per cent of patients at any one course. Stephens and his colleagues (1917—1919) found a somewhat similar rate in this disease

The treatments in Series 19 Q and 23 QA in our work are almost identical with the 'Standard Treatment of Malaria' adopted by the National Malaria Committee of the United States. This treatment is claimed to cure 90 to 95 per cent of 'malarial cases' (Bass, 1919, 1922). It may do so in the type 'malaria' case seen in general practice, but in our work with chronic benign tertian malaria it has not cured more than about 40 to 50 per cent.

The combination of alkali with quinine did not seem to have any markedly advantageous effect as compared with quinine alone in the treatment of this type of case.

Under the conditions of our tests, we have arrived at the conclusion that two-thirds of all patients suffering from chronic benign tertian malaria, will relapse after a course of quinine treatment similar to those used in this work.

#### (b) *Quinidine Treatment*

MacGilchrist (1915) states that as gauged by his results, this drug is distinctly inferior to quinine in eliminating parasites from the peripheral blood nor is it quite so good as regards relapses.

Acton (1920) reported the results of the treatment of 62 patients with this drug in doses of 20 grains daily for 21 days, with a relapse rate of only 37 per cent. He concluded that this alkaloid has a more selective action on *P. vivax* than had quinine and recommended its use for the treatment of benign tertian malaria.

Shapiro and Kligler (1924) treated 20 adults and 25 children with this drug, the dosage for an adult being 22 grains  $\times$  5 days, 10 grains  $\times$  30 days. The relapse rate was 26.6 per cent as compared with 28.5 per cent after a quinine course of 30 grains  $\times$  5 days, 10 grains  $\times$  30 days. They concluded that 'although the number of individuals observed is small, it is sufficient to indicate that quinidine is at least as satisfactory as quinine and is also perhaps a shade better, since smaller quantities of the drug yielded the same results as did quinine treatment'. In considering this result, it must be remembered that the patients

EXPLANATION OF PLATE LVII

Camera-lucida drawings of parts of *P barraudi* ( ♂ )

- Fig 1 Wing  
„ 2 Palp showing position of Newstead's spines (N)  
„ 3 Dorsal plate of pharynx  
„ 4 Buccal cavity showing teeth  
„ 5 Pompetta  
„ 6 Segments XII to XVI of antenna  
„ 7 Segments III and IV of antenna  
„ 8 Genitalia of male  
„ 9 Distal segment of superior clasper with an abnormal spine (S)



grams daily for 10 to 21 days in cases of chronic benign tertian malaria. This lower rate than seen in quinine treatment, he attributed to the proportions of quinidine and cinchonidine in the mixture. MacGilchrist (1915) thought that the good effects recorded after treatment with this drug were due chiefly to the presence of large amounts of cinchonine and to a less degree quinidine.

*Cinchona febrifuge* was tested by us in 110 cases with an average relapse rate of 73.1 per cent (maximum 74.0, minimum 68.7), which is 1.9 per cent higher than the average.

Its value in the treatment of chronic benign tertian malaria is almost equal to that of quinine when given in the same dosage. As recorded by many workers, it is slightly more liable to be followed by some nausea and vomiting than is quinine, but we have found this of little practical importance.

#### (f) *Summary of Results*

It is seen from the figures given in Table XI that the results obtained after the treatment of chronic benign tertian malaria by quinine, cinchonine and cinchonidine were almost the same. It would, therefore, seem that in therapeutic doses these three drugs have an almost equal value. It must, however, be noted that the dosage of quinine was greater than that of the other two alkaloids. Whether the latter alkaloids would give better results, if they could be tolerated, when administered in the same doses of quinine was not determined.

TABLE XI

Alkaloid used	Total patients	Number lost sight of	Number not relapsing	Number of relapses	PERCENTAGE OF RELAPSES				
					Observed	Possible maximum	Observed minimum	Average	Deviation from average
Quinine	667	66	184	417	69.4	72.4	62.5	68.0	-3.2
Quinidine	208	14	30	164	84.5	85.6	78.8	83.0	+11.8
Cinchonine	72	3	22	47	68.1	69.4	65.3	67.6	-3.6
Cinchonidine	107	24	23	60	72.3	78.5	56.0	68.7	-2.5
<i>Cinchona febrifuge</i>	110	25	19	66	77.6	82.7	60.0	73.1	+1.9
TOTAL	1,164	132	278	754	73.0	76.1	64.7	71.2	

# STUDIES IN MALARIA, WITH SPECIAL REFERENCE TO TREATMENT

## Part XI.

### THE CINCHONA ALKALOIDS IN THE TREATMENT OF BENIGN TERTIAN MALARIA

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ALTHOUGH much scientific work has been done on the effects of the different cinchona alkaloids in the treatment of benign tertian malaria, most of it has had reference to the production of clinical cures and few such experiments have been made, in which the patients were observed by systematic blood examinations for the occurrence of relapses after the termination of all treatment. The methods considered necessary for testing the effects of any treatment in the production of a permanent cure in malaria have been detailed in another paper of this series (Sinton, 1926)

The researches which have been carried out by different workers in recent years would indicate that, in as far as the reduction of fever and the rate of disappearance of parasites from the peripheral blood is concerned, the four common crystallizable alkaloids of cinchona bark, namely, quinine, quinidine, cinchonine and cinchonidine, have almost an equal value when administered in therapeutic doses to patients suffering from benign tertian malaria. If these same relative values should be found to exist when the alkaloids are tested for the production of a permanent cure of the disease, it would have a very important influence upon the present policy of cinchona cultivation. Quinine, however, still seems to be the drug of preference in the treatment of the malignant tertian type of the disease (Sinton, 1926a)

It can be seen from Table I that, of 25 known primary infections with *P vivax* contracted in nature and treated with quinine for 8 weeks, only one or 4 per cent relapsed. Among the 154 patients recorded in Table II, many of whom were fresh infections, the relapse rate due to *P vivax* was only about 30 per cent after a short course of quinine treatment (4 to 7 days), in spite of the fact that some of the relapses recorded may have been reinfections. Table III shows the results of the treatment of 44 patients under similar conditions to those recorded in Table II, but observed mainly by clinical methods, and in these the relapse rate was about 14 per cent.

If all these cases are grouped together, although not all fresh infections, there is a total of 251 patients with a relapse rate of about 24 per cent. This figure is very close to the 25 per cent obtained by James (1926) following the treatment of patients infected by mosquito bites experimentally.

On the other hand the relapse rate among 667 chronic infections treated with quinine averaged 68.0 per cent and for 1,164 patients treated by different cinchona alkaloids was 71.2 per cent (*vide* Table XI). These results are very similar to those recorded in the researches after the War.

These figures help to confirm the opinion of clinicians, strengthened by the results of the treatment of induced malaria, that the majority of fresh infections contracted in nature are more easily cured than are chronic relapsing ones.

### CONCLUSIONS

Under the conditions of our tests and with the dosage of the cinchona alkaloids used, the following conclusions were arrived at regarding the relapse rates after the treatment of infections due to *P vivax*.

(1) The percentage of relapses in a population suffering from fresh infections with *P vivax* is much lower than the percentage among a population composed of chronic relapsing infections only.

(2) The quinine and alkali treatment seems to be more efficacious than quinine alone in curing fresh infections, but the results in chronic infections showed no very marked benefit.

(3) The four chief crystallizable alkaloids of cinchona bark—quinine, quinidine, cinchonine and cinchonidine—showed almost an equal value in preventing relapse in chronic infections. Quinidine gave the worst result.

(4) No evidence was found to confirm the assertion that quinidine was more effective than the other alkaloids in the treatment of infections with *P vivax*.

(5) Cinchona febrifuge has a value which, for most practical purposes, seems equal to that of the pure alkaloids in the treatment of chronic infections.

Our thanks are due to the Indian Research Fund Association who provided the funds for this research and to the Director of Medical Services in India for the facilities which he has placed at our disposal.

results of each control series, but merely to refer to the number, etc., of the control treatment used and this can be consulted where necessary

The treatment was in all instances administered orally in the form of solutions, except in Treatment Nos CFA 11 and CF 12, where tablets were used. In no instance was it found necessary to resort to any other form of treatment except the oral one and no deaths have occurred during our work.

The prescriptions of the different mixtures used in treatment are given in Appendix A.

## RESULT OF THE TREATMENT OF FRESH INFECTIONS

### (a) *British Patients*

These patients were British soldiers who developed their first attacks of malaria after arrival in Kasauli. The infections had been contracted in other parts of India before they came to the hills. The results of the treatment of 38 such patients are recorded in Table I. These patients were treated at the same time as those recorded in Tables IV—X.

TABLE I

Series (a)	Treatment Grams of drug $\times$ days (b)	Total drug — days (c)	Number of patients	Number lost sight of (d)	Number of relapses (e)
1 QA	30 $\times$ 14, 10 $\times$ 42	840 — 56	14	0	1
1 (a) QA	30 $\times$ 14, 20 $\times$ 7	560 — 21	4	0	0
2 Q	30 $\times$ 14, 10 $\times$ 42	840 — 56	11	0	0
3 QD	20 $\times$ 28	560 — 28	1	0	0
4 CC	20 $\times$ 28	560 — 28	2	0	0
5 CD	20 $\times$ 28	560 — 28	2	1	0
6 CF	30 $\times$ 7, 20 $\times$ 21	630 — 28	4	0	2
		TOTAL	38	1	3

(a) 'Q' in all the tables means treatment with quinine sulphate, 'QD' with quinidine sulphate, 'CC' with cinchonine sulphate, 'CD' with cinchonidine sulphate, and 'CF' with cinchona febrifuge (Kinetum). 'QA', 'QDA' and 'CFA' show that the drug treatment has been combined with the use of alkali as described by Sinton (1923a, 1926a).

(b) This column gives the daily dosage of the alkaloid and the number of days during which each dosage was given in chronological order.

(c) This is the total amount of the alkaloid given in grams, divided by the time which elapsed before treatment was completed.

(d) These patients did not complete eight weeks observation by blood examination after the end of treatment.

(e) All relapses are due to *P. vivax* unless otherwise stated. The relapses were diagnosed by blood examination.

(9) Tablets of cinchona febrifuge were used in Series 11 CFA and 12 CF and the former series also received alkali

(10) Some of the mixtures used contained various flavouring materials to disguise the taste

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histories, it seemed very probable that a considerable proportion of the cases were fresh infections. The results of the treatment of these patients has already been described in another paper (Sinton, 1923a)

These patients were treated under conditions which did not preclude reinfection, so it is impossible to exclude this as a cause of some of the so-called 'relapses'. That reinfections were probably occurring is shown by the fact that some of the patients who were treated in the latter half of the year when infections with *P. falciparum* were rife, showed this parasite only at the time of 'relapse' (vide Tables II and III)

The results of the treatment of 154 patients is shown in Table II. Series 7 and 8 acted as controls to each other, while Series 9 to 12 ran concurrently and similarly controlled each other.

TABLE II

Series	Grams of drug $\times$ days of treatment.	Total drug — days of treatment	Number of patients	NUMBER OF RELAPSES			PERCENTAGE OF RELAPSES		
				BT (a)	MT (b)	Total	BT	MT	Total
7 QA	20 $\times$ 1, 30 $\times$ 3	110 — 4	43 (c)	12	0	12	27.9	0	27.9
8 Q	20 $\times$ 1, 30 $\times$ 3	110 — 4	45 (c)	18	0	18	40.0	0	40.0
9 QA	20 $\times$ 1, 30 $\times$ 5, 10 $\times$ 1	180 — 7	18 (d)	2	2	4	11.1	11.1	22.2
10 Q	20 $\times$ 1, 30 $\times$ 5, 10 $\times$ 1	180 — 7	14 (d)	3	3	6	21.4	21.4	42.8
11 CFA	30 $\times$ 4	120 — 4	18 (d)	3	4	7	16.6	22.2	38.8
12 CF	30 $\times$ 4	120 — 4	16 (d)	7	2	9	43.7	12.5	56.2
		TOTAL	154	45	11	56	29.2	7.1	36.3

(a) 'BT' means benign tertian malaria (*P. vivax*)

(b) 'MT' means malignant tertian malaria (*P. falciparum*)

(c) Patients treated at the season when few fresh infections with *P. falciparum* were occurring

(d) Patients treated at a time when fresh infections with *P. falciparum* were common

The relapse rate in these cases is higher than in the British series, but this is to be expected because (a) the course of treatment was shorter, (b) some

orange juice, given in certain groups Two hundred pigeons divided into five groups of forty were used, the diets given being —

Group No 1	Autoclaved rice
Group No 2	Autoclaved rice plus autoclaved dhal
Group No 3	Autoclaved rice, autoclaved dhal, plus 5 drops of cod-liver oil and 10 drops orange juice
Group No 4	Autoclaved rice, fresh dhal, plus 5 drops cod-liver oil and 10 drops orange juice
Group No 5	Mixed fresh grain (unhusked rice, wheat, moong, arhar dhal and cholam)

The rice or dhal was autoclaved at 120°C for half an hour

The dhal was a mixture of equal parts of the two pulses, moong dhal and arhar dhal

The rice was supplied in excess of appetite and dhal to an amount of six grammes per bird daily was available Cod-liver oil and fresh orange juice was given by hand Groups No 3 and 4 provide the exact contrast required in the sole difference of vitamin B content

The occurrences in regard to the pigeons in the five groups are shown in Tables I and II, and Chart 1 and may be summarized as follows —

*Group No 1*—Two birds escaped, leaving 38 for experiment Rapid and continuous fall of average weight until death of last bird on the 53rd day of the experiment

Deaths were due to —

Polyneuritis	15
Starvation	16
Other causes	7

*Group No 2*—Rapid fall of average weight to about the 30th day Fair degree of maintenance to 60th day Further rapid fall to 100th day Half the pigeons were dead by the 38th day All but two were dead by the 100th day

These survived to the 107th and 203rd day, respectively

Deaths were due to —

Polyneuritis	24
Starvation	7
Other causes	9

The two last survivors died of heavy tapeworm infection

*Group No 3*—The average body weight curve was very similar to that of Group No 2 One survivor on the 100th day lived to the 179th day, eventually dying of starvation

Deaths were due to —

Polyneuritis	23
Starvation	14
Other causes	3

the points used for determining 'relapses' in these artificially inoculated cases have left much to be desired. In some instances, the criterion of 'relapse' seems to have been the finding of parasites in the peripheral blood, when symptoms such as fever, etc., indicated that a relapse appeared imminent, while in other work 'relapse' was only recorded when both fever and parasites were present, irrespective of the presence of parasites on other occasions.

The systematic blood examinations, which were carried out for the determination of relapse rates by workers on naturally-infected malaria, do not seem to have been used as a routine, so the relapse rates recorded in therapeutic malaria are not strictly comparable with those obtained by blood examination in natural infections. The long periods over which many of the patients were observed clinically, tends to lessen this discrepancy. It does not, however, abolish it, for the relapses in natural malarial infections were diagnosed on the presence of parasites, irrespective of clinical symptoms, while in artificially induced malaria less stress seems to be laid on the presence of parasites in the absence of fever. Many of the cases recorded in this paper showed parasites in their peripheral blood without clinical symptoms. They were therefore recorded as relapses, which does not seem to be the rule in therapeutic malaria.

There is great difficulty in obtaining numbers of fresh malarial infections in the tropics under conditions in which the effects of treatment in producing a permanent cure can be scientifically observed along the lines advocated by Sinton (1926). For this reason the results obtained in Europe with fresh infections, treated under conditions precluding reinfection, are of great interest. The results of quinine treatment in the cure of fresh malarial infections produced by laboratory methods may be divided into two groups (a) those in which the patients were infected by blood inoculation, and (b) those in which the infection was conveyed by the bites of mosquitoes.

#### *(a) Effects of treatment in curing infections following blood inoculation*

Yorke and Macfie (1924) record sixty-one patients treated with a relapse rate of 1.6 per cent, following a treatment lasting only three days with 30 grains of quinine daily. Yorke (1925) in a later paper states that over 100 patients have now been observed after a similar treatment with a relapse rate of about 2 per cent.

Rudolf (1927) has collected the results of the treatment of 455 cases by various workers, in which the rate was only 2.2 per cent. Of 71 patients treated by this worker with a total of 200 grains of quinine in 17 days (20 grains  $\times$  3 days, 10 grains  $\times$  14 days), only one or 1.4 per cent were recorded as relapsing, while of 7 patients who received only a single dose of 20 grains of quinine, all relapsed.

Similar low percentages of relapses have been reported by other workers, and it would seem evident that malaria following the inoculation of infected blood is very easily cured in the great majority of patients by very short courses and small amounts of quinine.



TABLE I—*contd*

Day	Group 1	Group 2	Group 3	Group 4	Group 5
29	26	31	34	39	37
30	26	31	34	39	37
31	22	31	33	39	37
32	20	30	31	39	37
33	16	27	29	39	37
34	15	27	28	39	37
35	13	22	26	39	36
36	11	22	25	39	36
37	11	21	25	39	36
38	11	20	24	39	36
39	10	19	23	39	36
40	10	19	21	39	36
41	9	18	20	39	36
42	8	15	19	39	36
43	6	15	19	39	36
44	6	15	19	39	36
45	4	15	18	39	36
46	4	15	18	39	36
47	3	15	18	39	36
48	3	15	18	39	36
49	2	14	18	39	36
50	2	14	17	39	36
51	1	13	15	39	36
52	1	13	15	39	36
53		13	14	39	36
54		13	14	39	36
55		13	14	39	36
56		13	13	39	36
57		12	12	39	36

cure rate may be taken to represent fairly well that to be expected in the forms of benign tertian malaria seen in general practice in the tropics, where both fresh and chronic infections are being dealt with. The success of the American Standard treatment, which is claimed to cure about 90 per cent of cases of 'malaria' (Bass, 1922), is probably due to the admixture of fresh and chronic infections and also probably of some malignant tertian ones.

The disrepute into which quinine treatment fell, during and after the War, seems to be due to the fact that most of the work was carried out with chronic relapsing infections which, as will be seen later, are more difficult to cure, and not with both types of the disease as ordinarily seen in practice.

The patients treated with a short course of the quinine and alkali treatment gave better results than did those treated with a similar course of quinine only (*vide* Tables II and III). This has been discussed in another paper (Sinton, 1923*a*).

When the results of the treatment of fresh infections with *P vivax* are compared with those of chronic relapsing ones, the evidence is very strong that the former infections are more easily cured than the latter.

## RESULTS OF THE TREATMENT OF CHRONIC INFECTIONS

The results recorded here were all obtained from the treatment of British soldiers who had been sent for special treatment on account of chronic relapsing infections due to *P vivax*. The number of previous relapses recorded by each patient varied from 3 to 20 or more, with an average number of about 5 each.

The conditions under which these patients were treated and the methods of observation have been described in detail in other articles of this series.

Quinine, quinidine, cinchonine, cinchonidine and the mixture of these alkaloids, cinchona febrifuge, were tried. The mixtures used for oral administration are described in Appendix A.

### (a) Quinine Treatment

(1) *Quinine only*—Table IV shows the results of the treatment of 442 patients with quinine sulphate in solution. The duration of treatment was from 10 to 56 days and the total amount of the drug given varied from 240 to 840 grains.

Series 15 Q were controls for Plasmoquine treatment (Sinton and Bird, 1928). Series 16 Q and 17 Q controlled treatment. Nos 20 QA, 21 QA, 24 QD, 25 QD, 28 QDA, 29 QDA and 31 CD. Series 18 Q were controls for Nos 22 QA, 23 QA, 26 QD, 34 CF and 35 CFA, while No 19 Q was carried out at the same time as Nos 23 QA (*pro parte*), 27 QD and 32 CD.

The after histories of all the 19 cases 'lost sight of' in Series 17 Q was traced, and of these 8 had relapsed and 11 had no clinical history of relapse up to 6 months after the end of observation.

TABLE I—*concd*

Day	Group 1	Group 2	Group 3	Group 4	Group 5
87		5	5	39	35
88		3	4	39	35
89		3	4	39	35
90		3	4	39	35
91		3	4	39	35
92		3	4	39	35
93		3	3	39	35
94		3	2	39	35
95		3	2	39	35
96		3	2	39	35
97		3	1	39	35
98		3	1	39	35
99		2	1	39	35
100		2	1	39	35

*Group No 4*—Body weights well maintained after slight initial fall Final weight higher than initial weight One death from filariasis

*Group No 5*—Increasing body weight Five deaths from miscellaneous causes No case of polyneuritis

The food Chart 2 shows the stimulus to appetite provided by vitamin B in the case of Group No 4 in contrast with the rapid loss of appetite for starch in Groups No 2 and 3 in which the consumption of rice soon become very small although most of the dhal provided was eaten

The results in the case of all pigeons dying with polyneuritis have been examined in the light of McCarrison's work and we have made a diagnosis of beri-beri columbarum in a proportion of cases more or less on the lines adopted by him Detailed post-mortems were made in every case and the weights and the condition of organs registered While accepting McCarrison's statement of the post-mortem findings on which a diagnosis of beri-beri columbarum can definitely be made, we have found that the absolutely typical beri-beri heart may be present and at the same time certain of the other characteristic appearances absent For example, we have found that marked congestion of the liver was not frequent in our series Where the typical large degenerated heart was present along with some, but not necessarily all, of the other conditions we have made a

Series 20 QA and 21 QA were controlled by Series 16 Q and 17 Q respectively, while Series 18 Q controlled 22 QA, and 19 Q controlled 23 QA

As shown in Table V, a total of 245 patients were treated with this method and of these an average of 68.3 per cent relapsed. The 'after histories' of 17 of the 19 cases 'lost sight of' in Series 21 QA are available. Of these eight relapsed, while nine had no clinical symptoms of malaria up to the end of six months after completing observation by blood examination. There was no evidence that the alkali treatment was more effective in preventing relapse in chronic benign tertian malaria than quinine treatment alone.

TABLE V

Series	Grains of drug $\times$ days of treatment.	Total drug — days of treatment.	Total number of patients	Number lost sight of	Number not relapsing	Number of relapses	PERCENTAGE OF RELAPSES			
							Observed	Possible maximum	Observed minimum	Average
20 QA	20 $\times$ 1, 30 $\times$ 6, 10 $\times$ 7, 20 $\times$ 7	340 — 21	61	1	8	52	86.6	86.8	85.2	86.2
21 QA	30 $\times$ 14, 20 $\times$ 7	560 — 21	95	19	22	54	71.0	76.8	56.8	68.0
22 QA	30 $\times$ 7, 10 $\times$ 7, 30 $\times$ 7, 10 $\times$ 35	770 — 56	31	2	15	14	48.2	51.6	45.1	48.3
23 QA	30 $\times$ 14, 10 $\times$ 42	840 — 56	58	6	20	32	61.5	65.5	55.5	60.7
		TOTAL	245	28	65	152	70.0	73.4	62.1	68.3

A total of 667 cases of chronic benign tertian malaria were treated with quinine sulphate in solution with maximum doses of 30 grains daily for periods of from 21 to 56 days with an average relapse rate of 68.0 per cent (*vide* Table XI)



TABLE VII

Series	Grams of drug $\times$ days of treatment	Total drug — days of treatment	Total number of patients	Number lost sight of	Number not relapsing	Number of relapses	PERCENTAGE OF RELAPSES			
							Observed	Possible maximum	Observed minimum	Average
28 QDA	20 $\times$ 7, 17 $\times$ 7, 20 $\times$ 7	280 — 21	47	0	7	40	85.1	85.1	85.1	85.1
29 QDA	20 $\times$ 21	420 — 21	41	1	6	34	85.0	85.3	83.0	84.4
		TOTAL	88	1	13	74	85.0	85.2	84.1	84.8

The total number of patients treated with quimidine sulphate was 208 with an average relapse rate of 83 per cent (*vide* Table XII). In these chronic cases the alkali treatment did not seem to be any more effective in preventing relapse than quimidine without alkali.

(c) Cinchonine Treatment

In Table VIII are shown the results of the treatment of 72 patients with cinchonine sulphate. The average relapse rate was 67.6 per cent. This treatment was controlled by Series 19 Q, 33 CF and 32 CD.

TABLE VIII

Series	Grams of drug $\times$ days of treatment	Total drug — days of treatment	Total number of patients	Number lost sight of	Number not relapsing	Number of relapses	PERCENTAGE OF RELAPSES			
							Observed	Possible maximum	Observed minimum	Average
30 CC	20 $\times$ 28	560 — 28	72	3	22	47	68.1	69.4	65.3	67.6



they left our observation, while of the ten cases in Series 35 CFA five had not relapsed and four had

The average relapse rate among the 110 patients treated with cinchona febrifuge was 73.1 per cent

TABLE X

Series	Grains of drug $\times$ days of treatment	Total drug — days of treatment	Total number of patients	Number lost sight of	Number not relapsing	Number of relapses	PERCENTAGE OF RELAPSES			
							Observed	Possible maximum	Observed minimum	Average
33 CF	30 $\times$ 7, 20 $\times$ 21	630 — 28	70	3	17	50	74.6	75.7	71.4	73.9
34 CF	30 $\times$ 7, 20 $\times$ 24	690 — 31	20	12	1	7	87.5	95.0	35.0	68.7
35 CFA	30 $\times$ 7, 20 $\times$ 24	690 — 31	20	10	1	9	90.0	95.0	45.0	74.0
		TOTAL	110	25	19	66	77.6	82.7	60.0	73.1

#### THE COMPARATIVE VALUES OF THE DIFFERENT CINCHONA ALKALOIDS IN PRODUCING A PERMANENT CURE IN CHRONIC BENIGN TERTIAN MALARIA

Dymock, Warden and Hooper (1891) record that the Madras Government in 1866 appointed a Commission to test the relative efficacy of the four crystallizable alkaloids of cinchona bark in malaria. The tests were carried out clinically and the Commission placed the drugs in order of anti-malarial value as follows — Quinidine, quinine, cinchonidine and cinchonine, the first three being almost equal in value, while the last was less useful. A later report in 1880 gives cinchona febrifuge an almost equal value to the first three alkaloids. MacGilchrist (1915) says that 'the various cinchona alkaloids were tested clinically in the military hospitals in Algiers and at Rochelle (1858) and also in India (1870), and the conclusions come to were that quinine was the best and that, of the others, quinidine was the strongest and cinchonine was the weakest in anti-malarial power'. It must, however, be remembered that these investigations were carried out before the discovery of the malarial parasite, so it is almost certain that all the cases treated were not malaria, and that the results obtained take no cognisance of the three different forms of parasite. Another objection to these experiments is that the research was apparently concerned with the clinical effects of the drugs rather than their effect in preventing relapses. It would, however, appear to be





were apparently observed by clinical methods only and not by routine blood examinations, and also that the cases treated probably included a number of fresh infections

A total of 208 cases of chronic benign tertian malaria were treated in our work with an average relapse rate of 83.0 per cent (maximum 87.2, minimum 68.7), which is 11.8 per cent higher than the average of all the results in treatment by the cinchona alkaloids

The results obtained in the research reported here, do not bear out the view that quinidine is superior to the other alkaloids in the treatment of infection with *P. vivax*

#### (c) Cinchonine Treatment

Acton (1920) treated 14 patients with this drug in doses of 20 grains daily for 3 weeks and recorded a relapse rate of 57 per cent. He considers that the drug is not so valuable as quinine, because toxic symptoms developed in some of his cases

MacGilchrist (1915) in his 'Cinchona Derivatives Inquiry' states that 'according to the results obtained in this investigation, cinchonine is equal, if not superior, to quinine, whether in regard to eliminating parasites from the peripheral blood or in regard to effect upon crescents and relapses'. He did not find that the drug was more toxic than quinine and notes that the drug was used by a doctor in mistake for quinine, and in the same dosage, without ill-effects

In our research 72 patients were treated with this alkaloid and among these 67.6 per cent of relapses occurred, which is 3.6 per cent less than the average found for all the alkaloids

No toxic effects were observed and Fletcher (1926) also found that the drug was well tolerated in therapeutic doses. He attributes the supposed toxicity of the drug to the use of impure samples

The conclusions we arrived at were that this drug had a power equal to that of quinine in the cure of chronic benign tertian malaria

#### (d) Cinchonidine Treatment

Acton (1920) reports 46 patients treated with cinchonidine sulphate in doses of 21 grains daily for 20 days with a relapse rate of about 37 per cent. He considers that this drug as well as quinidine has a special selective action on *P. vivax*. MacGilchrist (1915) thinks that in anti-malarial action it is distinctly the weakest of the alkaloids

Among the 107 patients recorded in Table IX the relapse rate was 68.7 per cent (maximum 69.0, minimum 68.5), which is 2.5 per cent below the average for all the alkaloids

No evidence was forthcoming that the drug had any more marked action in curing infections with *P. vivax* than had quinine

#### (e) Cinchona Febrifuge Treatment

Acton (1920) treated 110 patients with this substance in tablet form and recorded an average relapse rate of about 48 per cent after treatments of 21

Our diagnosis of beri-beri columbarum has been made chiefly in regard to pigeons in heart categories, AI and A, but we have also included a few in category B when all the characteristic conditions except marked increase in weight were present. A few in Group A have been put down as 'almost typical' when certain of the other points were absent. It has not been found possible to draw a hard and fast line based on heart weight alone. The main details of the findings in the case of pigeons dying with polyneuritis are shown in Tables III, IV and V.

The weights of all organs have not been given but only those more important for diagnosis.

The results are summarized in Table VI.

*In Group No 1* three cases are classified as beri-beri columbarum, representing 20 per cent of the polyneuritis cases and 7.8 per cent of the total deaths.

*In Group No 2* there were 9 deaths classified as 'typical beri-beri,' this being 37.5 per cent of the cases. Of these the majority occurred early in the progress of the experiment and showed AI or A category hearts. Two occurring later had B category hearts but showed otherwise typical appearances. In this group we also find four cases to be 'almost typical.' Five are classified as 'atypical.'

*In Group No 3* four deaths are classified as 'typical beri-beri,' all occurring at an early stage of the experiment, three with AI and one with A category hearts. Two are classified 'almost typical.' The percentage of typical beri-beri is 17.4 per cent. Thirteen deaths in this group showed post-mortem appearances which were 'atypical,' these including five with A category hearts.

From these results our difficulty in classifying cases on heart weight only will be readily seen. While we classify many cases as atypical, we consider that some of them present appearances indicating a degree of the beri-beri condition. In the three groups we have classified three, six and four cases respectively as polyneuritis columbarum, these being of the starvation type with marked reduction in heart weight.

The difference in the comparative incidence of beri-beri in the pigeons of Group No 2 as contrasted with Group No 3 is not easy to explain. There is little difference in the progress of mortality in the two groups or in their weight curves or food intake. The only difference in their feeding was the use of cod-liver oil and orange juice in the latter group.

The total number of typical plus atypical cases is nearly the same in both groups, a higher proportion of atypical cases occurring in Group No 3. The deaths from starvation presented an average period of survival in the pigeons of Group No 2 of 41 days as compared with 63 days in Group No 3. Whether the administration of cod-liver oil and orange juice prolonged the life of the pigeons of Group No 3 resulting in a greater influence of the starvation factor it is not possible to say. So far as the effect of vitamin B is concerned, it is possible that our groups are too small for accurate comparison and these two groups might be taken together. The combined incidence will be 27.7 per cent of the total cases of polyneuritis diagnosed as typical beri-beri and 40.4 per cent

Quinidine was found to give a distinctly higher relapse rate than the other alkaloids tested. There was no evidence that it had a greater curative action in infections with *P. vivax* than had quinine.

Cinchona febrifuge treatment gave a slightly higher average rate than did quinine, cinchonine and cinchonidine, but showed a better result than quinidine.

#### COMPARISON OF THE RELAPSE RATES IN FRESH AND CHRONIC INFECTIONS

The opinion formed by many workers during the Great War was that, in fresh infections with *P. vivax*, a much smaller percentage of relapses developed after treatment than in the case of the chronic relapsing ones. On the other hand, some workers, as Acton, Curjel and Dewey (1921), thought that fresh infections were not more easily cured than chronic ones.

It has been the experience of most workers in the tropics that few patients, unless subject to discipline, will conscientiously carry out the long courses of quinine treatment usually recommended for the cure of malaria. Even patients under strict discipline make efforts, sometimes successful, to escape from the tedium of this form of treatment. It is also extremely common, especially among uneducated persons, for a patient to take treatment only while clinical symptoms are present and then to abandon all treatment until a relapse occurs. Yet, in spite of this, very many patients treated for primary infections with *P. vivax* seem to be permanently cured by such curtailed courses, in the absence of reinfection. Such cures probably represent that proportion of patients whom it has been found possible to cure by short courses of quinine after experimentally-induced infections. If a considerable proportion of patients suffering from primary attacks were not cured by such short or incomplete courses, and relapsed subsequently, the number of malarial patients in tropical countries would be even greater than at present.

Although the view that fresh infections were more easily cured was believed by many workers, yet little scientific proof was forthcoming, because, until the introduction of malaria in the treatment of mental diseases, almost all the systematically controlled researches into the anti-relapse value of the cinchona alkaloids had been conducted with chronic relapsing infections. This was necessary on account of the great difficulty in obtaining fresh infections in sufficient numbers under conditions suitable for carrying out careful work in the absence of reinfection.

As can be seen from the results obtained in this paper, such chronic infections are not comparable to fresh infections in their reaction to treatment. The former probably represent those patients who were inadequately treated during their primary infections or represent that minority which it has been found in experimental malaria, are not easily cured of the disease.

James (1924), Manson-Bahr (1924) and Yorke (1925) all think that the difference in the susceptibility of induced malaria to quinine treatment has something to do with the fact that one is dealing with primary infections instead of with chronic relapses.

as 'typical' or 'almost typical' The incidence of beri-beri in proportion to total pigeons under experiment will be 23.7 per cent for these two groups as compared with 7.9 per cent for Group No. 1

The results with Groups No. 2 and No. 3 correspond with those of certain of McCarrison's groups showing a high incidence of beri-beri columbarum, and it is noted that in his groups of highest incidence the dietary used contained a considerable proportion of protein. This was a feature of the diet used in the case of Groups No. 2 and No. 3. The incidence is much higher than on diets of pure rice of low protein content from polishing or rendered further deficient by washing or autoclaving.

Our findings in this series agree with McCarrison's in the recognition of a definite pathological condition in pigeons presenting the same features as are found in fatal human beri-beri cases and produced in one group by a dietary differing from the control series in the single point of the absence of the antineuritic fraction of vitamin B. As will be seen in a later section of this paper the same condition has been also produced on a diet in which there is a deficiency but not a complete absence of this vitamin fraction. The more frequent observation of the typical appearances of beri-beri columbarum has probably been hindered by the extent to which pigeons not forcibly fed will have the characteristic changes modified by the effect of starvation, in the absence of the stimulus to appetite which vitamin B affords.

### SUMMARY AND CONCLUSIONS

1. The condition described by McCarrison under the name of beri-beri columbarum has been observed in pigeons on diets of rice, and of rice and dhal, deprived of the antineuritic fraction of vitamin B by autoclaving.

2. The post-mortem changes were characteristic, especially in the heart in which exactly the same changes were found as in human beri-beri.

3. While the post-mortem conditions were typical in some cases, in others the characteristic features were masked by a starvation factor in the causation of death.

4. The highest incidence of beri-beri columbarum occurred on a diet of autoclaved rice and dhal, with a high protein content.

5. The only factor concerned in the production of beri-beri columbarum was deprivation of the antineuritic vitamin. This was shown by its occurrence when autoclaved dhal was used and its absence when the same dhal was given in the fresh state.

## SECTION 2

### REFECTION IN PIGEONS

#### *Its spontaneous occurrence on a diet of autoclaved rice and autoclaved dhal*

From an examination of Chart 1, showing the average weights of the pigeons of the five groups of the first experiment, it will be seen that while the weights of the pigeons of Group No. 1 fell rapidly and continuously until all

## APPENDIX A

## PRESCRIPTIONS OF MIXTURES USED IN TREATMENT

- (1) Series 16 Q and 17 Q were treated with the following mixture —

Quinine sulphate	Grains 10
Acid sulphuric dil	Minims 10
Water to	Ounce 1

- (2) Series 7 QA, 8 Q, 9 QA, 10 QA, 13 QA, and 14 QA were given a mixture similar to No 1 but magnesium sulphate, 60 grains, was added to each ounce

The QA series received in addition doses of the following mixture before each dose of quinine —

Sodium bicarbonate	Grains 90
Sodium citrate	Grains 45
Calcium chloride	Grains 5
Water to	Ounces 2

The full details of the alkaline treatment are given by Sinton (1926a)

- (3) Series 18 Q, 19 Q, 20 QA, 21 QA, 22 QA and 23 QA were given the following mixture —

Quinine sulphate	Grains 10
Acid citric	Grains 25
Magnesium sulphate	Grains 60
Water to	Ounce 1

The QA series also received alkali

- (4) Series 15 Q received a mixture similar to No 3 but without magnesium sulphate, and with 12 grains of quinine to each ounce

- (5) Series 24 QD and 25 QD were treated with

Quinidine sulphate	Grains 10
Acid sulphuric dil	Minims 10
Water to	Ounce 1

- (6) Series 26 QD, 27 QD, 28 QDA and 29 QDA received

Quinidine sulphate	Grains 10
Acid citric	Grains 25
Magnesium sulphate	Grains 60
Water to	Ounce 1

The QDA series also received alkali

- (7) In Series 30 CC, 31 CD and 32 CD a mixture similar to No 5 was given, in which quinidine sulphate was replaced by cinchonine sulphate or cinchonidine sulphate in the same dosage

- (8) In Series 33 CF, 34 CF and 35 CFA the same mixture as in No 5 was used, except that quinidine was replaced by cinchona febrifuge in the same amounts. The mixture was strained through lint to obtain a nicer preparation. Series CFA also received alkali

# CHART 2

Average Daily Food Consumption of Group Nos 2, 3 and 4  
[In four-day periods]

Day of experiment

20

40

60

80

100

30

20

10

0

30

20

10

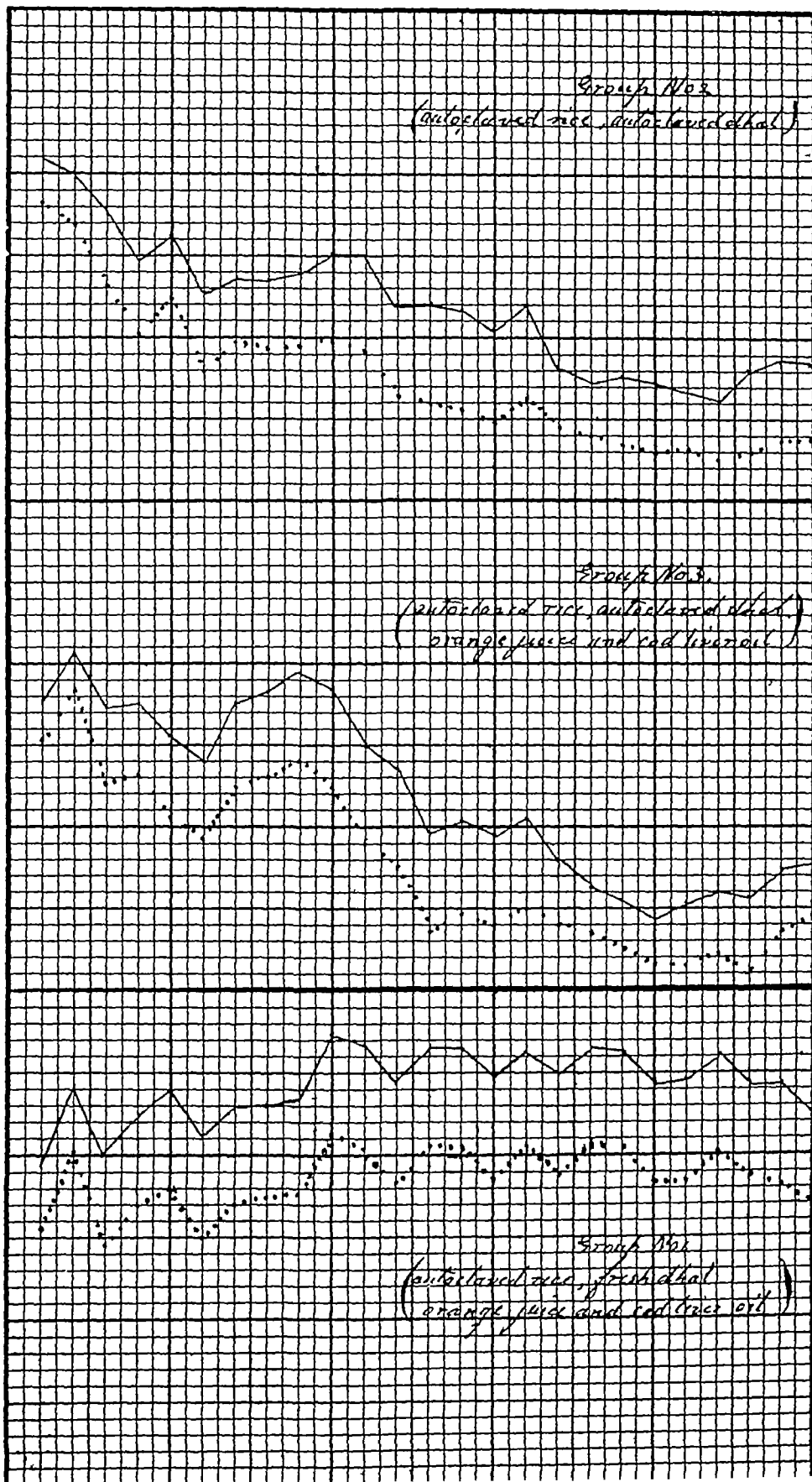
0

30

20

Food  
in grms 10

0



Total food -----

# OBSERVATIONS ON VITAMIN B DEFICIENCY IN PIGEONS (INCLUDING THE OCCURRENCE OF REFLECTION)

BY

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AND

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THE series of experiments to be described was commenced in the first place for the purpose of observing the incidence of the condition which McCarrison (1928) had designated 'beri-beri columbarum,' in pigeons fed on certain natural items of Indian dietary deprived of the antineuritic fraction of vitamin B by the action of heat. The observations were later extended to the effect of cooking on two commonly used food-stuffs which have frequently been used as accessories to a diet with the object of remedying any deficiency in vitamin B. During the course of the first experiments a phenomenon which we believe to be that which Fredericia (1927) and his collaborators have called 'reflection' was observed and this formed a further study. We have accordingly divided this paper into three sections dealing with —

- (1) Observations on beri-beri columbarum
- (2) Observations on 'reflection'
- (3) The effect of cooking on the antineuritic value of dhal and atta

The occurrences in certain of the experiments have a bearing on two or more of our headings and will be discussed in more than one section.

## SECTION 1.

### OBSERVATIONS ON BERI-BERI COLUMBARUM

#### *Experiment No 1*

The first experiment was carried out with groups of pigeons fed on natural staple Indian foods and arranged to show contrasting groups with differences only in regard to deficiency of the antineuritic fraction of vitamin B, a sufficiency of protein being supplied, and other vitamins, in the form of cod-liver oil and



*Group No 3*—The pigeon which did not show refection on segregation died 13 days later from starvation

One pigeon which lost the condition after 5 days died from polyneuritis 11 days after segregation

Three remained refected for 4, 7 and 9 days, respectively and died on these days, one from starvation and two from polyneuritis

Three remained refected throughout, dying from starvation on the 87th, 93rd and 97th days of the experiment

One with short periods of refection died from starvation on the 92nd day

One pigeon after a prolonged period of refection followed by periods of irregularity of the condition survived to the 179th day, dying of starvation

As the condition was not observed until the 65th day, we lack certain information as to the period for which refection had been present which we might correlate with the subsequent history of the different pigeons

Fredericia has shown that the fæces of refected rats can replace vitamin B to a certain extent and also that the refected condition can be transmitted to other rats by feeding them with refected fæces

Before segregation it was possible for our pigeons to have access to each other's fæces and possibly to obtain a certain amount of protection by eating fæces of others showing the condition, or even to acquire refection in this way In Chart 1 a marked fall in the average weight of the pigeons of Groups No 2 and No 3 is seen to follow segregation on the 65th day and in Charts 2 and 3 the diminished food consumption is also seen This would suggest that, as is also shown by the further histories of some of the pigeons, refection was of a temporary nature and in the case of others it would appear that, although judging only by the starch content of the stools, the condition remained present, it was not of high protective value We have, however, two pigeons which, showing typical starchy fæces over prolonged periods, lived for 6 to 7 months on a diet entirely free from the antineuritic fraction of vitamin B, dying eventually of starvation and worms, respectively, without symptoms of polyneuritis These two birds both showed periods of marked rise in body weight during part of the time when the refected condition was present

Of the pigeons which died between the 80th and 100th days showing well marked refection during the whole period of observation only one showed polyneuritis, of the atypical form

From the observation of this series of pigeons it would appear that refection in pigeons on the diet used was an occasional occurrence, not always persistent, and not always of high protective value In a few cases its presence prolonged life and was associated with increase in weight Even when well established it was not in every instance capable of ensuring complete maintenance

From want of necessary facilities we have been unable to carry out extensive chemical tests on the fæces of the pigeons which we considered to show refection, to determine whether the condition observed by us is exactly the same as described by Fredericia and by Roscoe

TABLE I

*Daily number of pigeons surviving in each Group*

Day	Group 1	Group 2	Group 3	Group 4	Group 5
1	38	40	40	40	40
2	38	40	40	40	40
3	38	40	40	40	40
4	38	40	40	40	40
5	38	40	40	40	40
6	38	40	40	40	40
7	38	40	40	40	40
8	38	39	40	40	40
9	38	39	40	40	40
10	38	39	40	40	38
11	37	39	40	40	38
12	36	39	40	40	38
13	36	39	39	40	38
14	36	39	39	40	38
15	36	39	39	40	38
16	35	39	39	40	38
17	34	38	39	40	38
18	34	38	39	40	38
19	34	38	39	40	38
20	33	38	39	40	38
21	31	37	39	40	38
22	30	37	38	39	37
23	29	36	37	39	37
24	29	36	37	39	37
25	29	36	36	39	37
26	26	34	36	39	37
27	26	34	35	39	37
28	26	32	34	39	37



TABLE I—*contd*

Day	Group 1	Group 2	Group 3	Group 4	Group 5
58		10	12	39	36
59		10	12	39	36
60		10	10	39	36
61	.	10	10	39	35
62		10	10	39	35
63		10	10	39	35
64	.	10	10	39	35
65		10	10	39	35
66		8	10	39	35
67		8	10	39	35
68		8	10	39	35
69	..	8	10	39	35
70	.	8	9	39	35
71		7	9	39	35
72	.	7	9	39	35
73		6	8	39	35
74	.	6	8	39	35
75	.	6	7	39	35
76	.	6	7	39	35
77		6	6	39	35
78		6	5	39	35
79		6	5	39	35
80		6	5	39	35
81		6	5	39	35
82		6	5	39	35
83		6	5	39	35
84		6	5	39	35
85		6	5	39	35
86		5	5	39	35

on a diet of autoclaved rice with or without the addition of refected fæces showed any evidence of refection

Of the pigeons in Group No 7 there were seven which showed no evidence of refection or only a short period of passing starchy fæces. One showed refection present for 20 days, the condition being lost later on and death occurring from polyneuritis. Two pigeons showed refection well established on the 34th and 29th days respectively, one surviving to the 110th day and the other to the 147th day. Death of both eventually occurred from polyneuritis of the starvation type.

In Group No 9 seven pigeons showed no evidence of refection or only slight intermitted periods of the passage of starchy fæces. One developed refection on the 21st day and the condition persisted to the 46th day when death occurred from starvation. In two others refection was established on the 15th and 34th days, respectively. In one of these which survived to the 77th day, there was a short period of loss of refection and in the other which survived to the 95th, the condition remained established throughout. Both lost their refection for two or three days before death.

Death in each case was due to polyneuritis of the starvation type with extremely small heart.

This series shows that refection did not occur on a diet of autoclaved rice and was not transmissible by feeding with refected fæces on this diet. It shows that, as in the first experiment, refection occurs spontaneously on a diet of autoclaved rice and autoclaved dhal (Group No 7) but a comparison of the results in Groups No 7 and No 9 does not indicate that feeding with refected fæces has any influence on its occurrence. It would appear that the condition is not readily transmissible to adult pigeons.

#### *Occurrence of Refection on a Diet showing some degree of Vitamin B deficiency*

In Section 3 of this paper an experiment on the antineuritic value of certain articles of Indian dietary in the cooked state is described. In the course of the experiment, refection was looked for and the results obtained may suitably be discussed in this section.

The cooked foods added to the diet of autoclaved rice were cooked dhal and cooked atta (coarsely milled wheat flour).

Two groups of 10 pigeons each received the cooked dhal to the extent of 6 grammes daily, given by hand, and one group of 10 pigeons received the same quantity of cooked atta. In each of the groups receiving dhal two pigeons died of polyneuritis (one death from beri-beri and three from the atypical form) thus indicating a deficiency of the antineuritic fraction. In the first of these groups (Group No 10) refection was not looked for until the 32nd day when it was found in all of the pigeons.

One pigeon lost the condition on the 42nd day and died on the 48th day from polyneuritis. Another lost its refection on the 55th day and died on the 64th day from polyneuritis. Of the remaining eight pigeons two showed periods





TABLE III

Details of *polyneuritis* cases of Group No 1

Pigeon No	Days under ex- periment	Initial weight	Final weight	Heart weight	Heart category	Characteristic heart changes	Hydropericardium	Other effusions	Liver weight	Spleen weight	Adrenal weight	Echymoses of upper intestine	Polyneuritis type	Typical beri-beri colum- barum
140	30	270	140	3.95	AI	Typical	—	+	5.01	0.21	0.08	+	A	Typical beri-beri colum- barum
439	33	270	150	3.01	A	"	—	—	4.95	0.29	0.18	+	C	Do
366	41	295	190	3.85	A	"	+	—	5.51	0.29	0.15	+	C	Do
445	19	310	170	2.85	B	Atypical	—	+	5.15	0.39	0.06	—	B	Atypical
309	25	240	140	2.97	A	"	—	+	5.00	0.26	0.08	+	B	Do
408	26	310	170	2.95	B	"	—	—	5.02	0.29	0.10	+	B	Do
358	30	280	130	2.85	B	"	—	—	4.09	0.20	0.12	+	C	Do
210	30	320	200	3.06	B	"	—	+	7.08	0.18	0.09	+	A	Do
189	33	270	130	2.90	B	"	—	—	3.85	0.29	0.08	—	B	Do
407	33	320	150	3.05	B	"	—	+	4.00	0.15	0.12	+	C	Do
334	35	250	140	2.56	B	"	—	—	4.00	0.18	0.12	+	B	Do
196	42	350	180	2.95	B	"	+	—	5.21	0.15	0.12	+	B	Do
233	26	280	160	2.01	C	—	—	+	6.05	0.25	0.08	—	B	Starvation type of poly- neuritis columbarum
376	36	320	170	2.01	C	—	—	—	4.40	0.12	0.09	+	B	Do
207	45	270	160	2.07	C	—	—	+	5.84	0.11	0.10	—	B	Do



We have not been able to determine the exact parts played in the process by the dhal starch and by the rice starch respectively, but the former appears to be necessary for its establishment in adult pigeons

### SUMMARY AND CONCLUSIONS

1 A condition has been observed in adult pigeons, characterized by the passage of starchy faeces, on a diet deprived of the antineuritic fraction of vitamin B by autoclaving, which is considered to be the same as that described by Fredericia and his collaborators as occurring in young rats on a diet deprived of both fractions of vitamin B, and called by them 'refection'

2 Refection occurred spontaneously in a small proportion of pigeons fed on autoclaved rice and autoclaved dhal. It did not occur on a diet of autoclaved rice and fresh dhal or on a diet of mixed fresh grain which prevented polyneuritis

3 The condition had no relationship to a high starch intake

4 In adult pigeons the presence of refection appeared to prolong life in some cases and to assist in maintenance of body weight to a certain extent. It did not appear to be as effective a protective process in adult pigeons as in young rats. In the case of two pigeons its presence was associated with prolongation of life to six and seven months respectively on a diet deprived of the antineuritic vitamin

5 Attempts to transmit the condition to adult pigeons by feeding with refected faeces were apparently not successful

6 Refection was not found to occur on a diet of autoclaved highly milled rice alone but occurred in a proportion of pigeons in all experiments when autoclaved dhal was added. The starch of dhal appears to be favourable to its occurrence

7 On a diet of autoclaved rice plus cooked dhal which, from the occurrence of polyneuritis in 20 per cent of the pigeons showed evidence of some degree of deficiency of the antineuritic fraction, refection was well marked in 80 per cent of the birds, which survived well maintained to the 150th day when observation was stopped. The regularity of its appearance suggests that the starch of dhal in the cooked state is more favourable to the occurrence of refection than when autoclaved. The successful maintenance on this diet may be due to the supplementing of a deficiency of the antineuritic fraction while with an autoclaved diet the whole requirements have to be met

### SECTION 3

#### THE EFFECT OF COOKING ON THE ANTINEURITIC VALUE OF DHAL AND ATTA

Dhal (a name given to various pulses) and atta (a coarsely milled Indian wheat flour) are common articles of Indian dietary used to varying extent in different parts of India

Atta is a staple food in northern India where beri-beri is rarely seen and is little used in the southern parts of the country or in Burma where the staple food

Pigeon No	Days under ex- periment.	Initial weight.	Final weight.	Heart weight.	Heart category
327	20	230	150	3.9	A
289	27	280	160	3.81	A1
416	28	300	160	3.95	A
100	32	240	140	3.40	A1
406	34	280	220	4.53	A1
118	34	290	180	3.99	A1
352	37	270	180	2.90	A
220	65	310	190	3.11	B
316	70	310	190	3.05	B
454	36	310	190	2.45	B
364	41	290	160	2.99	B
199	56	360	190	2.95	B
265	85	310	180	3.31	A
438	25	300	160	3.01	B
365	27	320	180	3.05	B
143	34	260	140	2.71	B
391	34	310	180	3.09	B
264	87	300	180	3.21	B
378	34	260	150	2.01	C
305	41	310	170	2.01	C
25	50	250	150	1.95	C
410	57	260	140	1.53	C
306	65	320	190	2.35	C
212	72	340	170	2.51	C



diagnosis of beri-beri columbarum. In other cases we have diagnosed beri-beri when there was no very marked increase in actual weight of the heart although its appearance and the degenerative changes present were characteristic and the post-mortem appearances in other organs were typical. We have classified our findings as —

- (1) Typical beri-beri columbarum
- (2) Almost typical beri-beri columbarum
- (3) Atypical cases
- (4) Polyneuritis columbarum

The first two groups would probably include many classified by McCarrison as true beri-beri although perhaps his classification was more rigid than ours. Most of the third group would correspond to his intermediate beri-beri cases. We have had abundant opportunities of checking our findings in regard to beri-beri columbarum by direct comparison with post-mortems in human cases. We have been able to demonstrate to assistants and students the exact correspondence between the human and the pigeon heart in cases of beri-beri. The characteristic heart in man presents all the same points as McCarrison had demonstrated in the pigeon. The heart is big, often heavy, broad with dilated auricles and often also dilated ventricles. The muscle is soft, pale and flabby and the heart flattens out when placed on the table. There is marked loss of fat, especially noticeable at the auriculo-ventricular junction, although this is not constant. Oedema in this situation is occasionally found. The superficial vessels are engorged and petechial hæmorrhages and ecchymoses on the surface are usually present. Fatty degeneration of the myocardium is frequent. A slight degree of hydro-pericardium is often found. The findings in fatal human cases are fairly constant, but in a proportion of cases the degree to which the different changes are present varies considerably. The proportion of characteristic hearts in human cases is greater than in deficiently fed pigeons, probably from the fact that the starvation factor which is likely to enter into these cases will affect the size of the heart and other organs. In addition to observation of the various degenerative changes in the hearts of our pigeons we have classified the hearts on their weight in proportion to initial body weight, adopting the groups which Mr Sundararajan has used in his statistical analysis of McCarrison's (1928) results.

His categories are —

A—Heart weight greater than the mean of control pigeons

B—Heart weight between the mean of deficiently fed pigeons and the mean of controls

C—Heart weight below the mean of deficiently fed pigeons

To these categories we have added a fourth category—

AI—Heart weight greater than the maximum of control normally fed pigeons

This group emphasizes a very definite feature of beri-beri

The insufficiency of vitamin B in the cooked dhal as compared with the adequate amount contained in an equal quantity of cooked atta is probably due to the difference in the methods of cooking used. The prolonged boiling necessary to cook dhal properly will be likely to reduce the vitamin content more than the short period of cooking used for atta which is very quickly cooked.

In our experiments dhal and atta have been used to the extent of 20 per cent and 25 per cent of the total food intake. McCarrison has found the dhal added to a diet of washed milled rice to the extent of 8 per cent did not prevent polyneuritis and that high percentages of deaths from beri-beri columbarum occurred on the diet. He also instances the occurrence of human beri-beri on a diet containing 7 per cent to 10 per cent of dhal. Where an Indian dietary includes dhal the quantity used is seldom more than 4 or 5 ounces daily and its proportion to the total dietary is much lower than 20 per cent.

Dhal is unsuitable for addition to a rice dietary in very large quantities, and we have shown that added to a deficient rice dietary in the cooked state it will not prevent polyneuritis even at 25 per cent of total diet. Atta, on the other hand, can be used in much larger quantity and may form the greater part of a dietary. The Indian Army Field Service ration includes 1½ lbs of atta and 4 ounces of dhal. McCarrison has noted these points as to the habits in use of these foods and to his observations we have been able to add a quantitative test showing the deterioration of the antineuritic fraction of vitamin B during the cooking of dhal which renders it of low value even when used in large quantities. We come to the same conclusion that of foods commonly used in India atta is the best available preventative of beri-beri, not only from the general considerations as to the quantity which may suitably be used but also from actual trial in the cooked state.

#### SUMMARY AND CONCLUSIONS

1 The addition of dhal and atta in the cooked state, as ordinarily prepared for human consumption, to a diet of autoclaved rice to the extent of 25 per cent of the total food intake showed marked differences in the antineuritic value of these two articles of food.

2 20 per cent of pigeons receiving cooked dhal died of polyneuritis and the remainder developed the protective phenomenon of refection which is associated with a deficiency of vitamin B. All of the pigeons receiving cooked atta remained alive and well and none showed refection.

3 There is definite evidence of deficiency of the antineuritic factor in the dhal, due to cooking, a similar amount of fresh dhal having been found to be fully protective.

4 The amount of dhal given was much greater in proportion than is normally used in Indian dietaries or can satisfactorily be added to a rice diet.

5 The atta was used in a proportion which is less than it often forms in a diet.

TABLE VI  
Summary of deaths and findings in Groups No 1 to No 4 over 100 day period

Group	No of Pigeons in Group	Deaths from			Survived	HEART CATEGORIES OF POLYNEURITIS CASES				DIAGNOSIS			
		Polyn neuritis	Starvation	Other causes		AI	A	B	C	Typical beri-beri	Almost typical beri-beri	Atypical cases	Polyn neuritis starvation type
1	38	15	16	7		1	3	8	3	3		9	3
2	40	24	7	7	2	5	3	10	6	9	4	5	6
3	40	23	13	3	1	1	10	8	4	4	2	13	4
4	40	..		1	39								..
5	40			5	35								

# FURTHER OBSERVATIONS ON MALARIA IN COORG

BY

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IN a previous paper (McCombie Young and Baily, 1928) the results of a malarial survey of Coorg Province in June 1927 were recorded. These observations were incomplete, owing to our study of the Anopheline breeding grounds having been cut short by the onset of the monsoon rains, and practical measures had still to be devised. The following observations are in continuation of those already recorded.

Survey operations on the Anopheline breeding grounds in Mercara were again commenced in March, 1928.

The season was in some respects, an abnormal one, unusually heavy rain having fallen in the spring months, and the drains and water-courses had been scoured out, and Anopheline breeding in them retarded.

During March, the predominant species was *A. jeyporiensis* but *A. maculipalpis* and *A. majidi* were also common, and *A. subpictus*, *A. vagus*, *A. barbirostris*, and *A. jamesi* were collected.

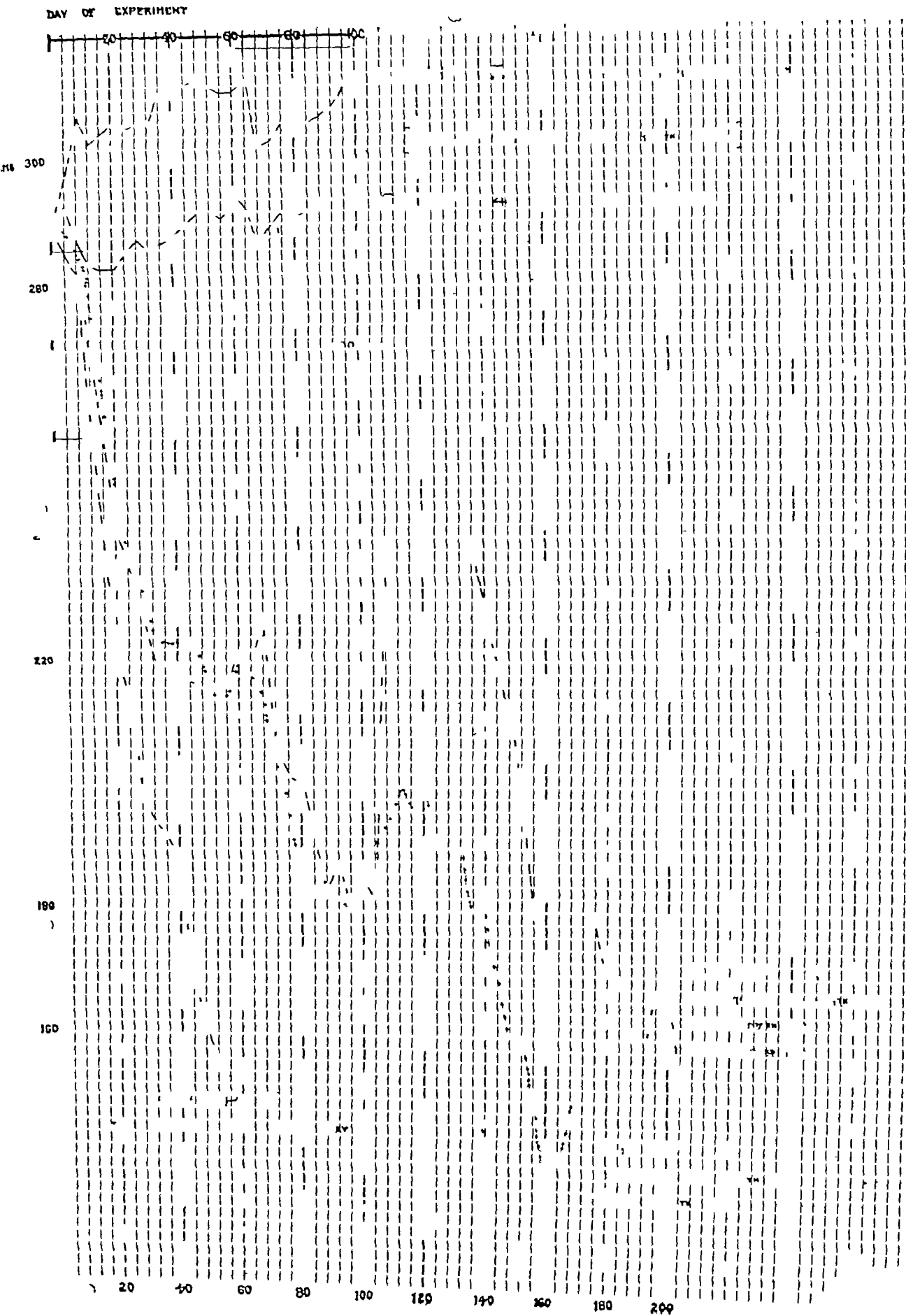
The cross drains and the stone-paved drains in Brahman Valley and elsewhere, which in May 1927 were breeding *A. listoni* in profusion, were in March 1928 devoid of this species, and contained only *A. jeyporiensis*.

By the third week of April, *A. listoni* and *A. maculatus* began to appear in the situations in which last year they had been found in abundance, i.e., in stone-paved, artificial drains, and in the cross drains. *A. maculatus*, of which in May last year only one specimen was collected was later very plentiful.

By the end of April, *A. jeyporiensis* had almost entirely disappeared, being replaced by the above-mentioned species and *A. culicifacies*, in what one may term 'domestic' water, while *A. Maculipalpis* and *A. majidi* were the Anopheline fauna of the 'jungle' waters.

# CHART 1

Showing Average Weight of Pigeons in Each Group  
[Four-day periods]





*A. subpictus* and *A. maculipalpis* were collected in recent rain-water pools in a paddy field, a grass-covered drinking water tank yielded larvæ of *A. barbirostris*, a cattle shed yielded adult *A. subpictus*, *A. histom*, *A. jeyporiensis* and *A. maculatus*.

The Dibidi lines are situated at a lower level and are surrounded by thick vegetation. A hill stream, running over a stony sandy bed and shaded by trees, lies below them, in the valley of which is situated the pulp house.

This stream and a tributary yielded the larvæ of *A. histom*, *A. maculatus*, *A. culicifacies* and *A. barbirostris*.

The water-supply of the lines is from wells, one being a large pucca well recently constructed to supply drinking water to the lines.

From this well, larvæ of *A. maculatus*, *A. leucosphyrus* and *A. barbirostris* were collected.

There are other small excavated springs which are at present used for drinking water. From one of these, *A. maculatus* and *A. leucosphyrus* larvæ were collected.

The spleen rate here is still high, out of 15 resident children, of poor and stunted physique, 12 had enlarged spleens.

Only one adult mosquito (*A. histom*) was collected in the lines.

The cause of the malariousness of this estate would appear to be, as was suspected last year, the proximity to the stream in which three of the most notorious malaria-carrying mosquitoes are breeding, viz., *A. histom*, *A. maculatus* and *A. culicifacies*.

The somewhat unexpected find of *A. maculatus* in the well suggested that the kutchha wells should be closed, and that the well, to which a pump is fitted, should have a closed well head.

The circumstances of this estate suggested that in selecting the site for the coolie lines of coffee estates, a valley with a stream running through it should be avoided and the top of a hill with open ground around it, free from jungle, should be preferred, despite the inconvenience of the distance of such a site from the pulp house which on account of the need for water, usually be situated in the valleys.

Jamboor coffee estate last year had a 100 per cent spleen rate among 20 resident children, and 91 per cent among 23 resident adult labourers.

Since then, the management of this estate had given considerable attention to the hygiene of its labour force, a hospital had been opened, and the health and comfort of the labour force had been studied.

A stream adjoining the lines running through coffee had been periodically sprayed with larvicide, and no Anopheline larvæ could be collected in it.

A similar but untreated stream which supplies the pulp house with water was also devoid of larvæ.

On exploring this stream, it was found to be full of the decaying fruit of a wild fig. A single uncontrolled experiment seemed to indicate that a decoction

were dead, there was a period between about the 30th and 60th days during which the weights of the pigeons of Groups No 2 and No 3 was maintained at a fairly constant level after the initial rapid drop. There did not at first appear to be any explanation of this period of maintenance, but while the observations were in progress, we received the articles by Fredericia (1927) and his collaborators and by Roscoe (1927) describing the phenomenon in rats which the former author has designated 'refection'. The possibility of the presence of this condition was at once investigated and an examination of the faeces of all birds under experiment was made on the 60th day.

At this time there were ten survivors each in Groups No 2 and No 3 and a total of 74 in Groups 4 and 5.

The majority of the pigeons surviving in Groups No 2 and No 3 showed the presence of faeces which on microscopic examination appeared to be almost entirely composed of unaltered starch grains. None of the pigeons in Groups No 4 and No 5 showed this condition. The food consumption of these groups is shown in Charts 2, 3 and 4 and it will be seen that the total intake of starch was much less in Groups No 2 and No 3 than in the Group No 4. All pigeons of Group 1 were dead at the period of this observation.

Up to this time the pigeons had been kept in cages each containing ten birds numbered by rings, and their food consumption estimated on the average of the groups. To study the condition further, each pigeon was isolated in a separate cage on the 64th day and their faeces studied daily.

At the examination on the 65th day, eight out of the ten survivors of Group No 2 and nine out of the ten in Group No 3 showed the typical starchy faeces. The average starch content of the faeces was 38 per cent.

In describing the further progress of these pigeons we shall for convenience use Fredericia's term 'refection' for the condition found, giving our reasons for considering that we are dealing in some degree of the same condition later.

The further progress of these surviving pigeons of Group No 2 and No 3 is best seen in Chart 3 in which we have represented graphically the periods during which each pigeon passed the typical starchy faeces. The occurrences in regard to these groups from the 65th day onwards may be summarized as follows —

*Group No 3* The two pigeons not showing refection died on the same day. In two others, refection was lost at once and death occurred 4 and 6 days later from polyneuritis.

Three with well marked refection lived to the 85th, 87th days respectively, dying of worms, polyneuritis and starvation.

One with irregular appearance of the condition lived to the 98th day dying of worms, and another showing periods of refection and finally losing the condition died to the 107th day.

One with well marked refection over long periods with shorter intervals of irregularity, lived to the 203rd day eventually dying of a heavy tapeworm infection.

# SOME MEDICINAL PLANTS GROWING IN THE HIMALAYAS.

## II

BY

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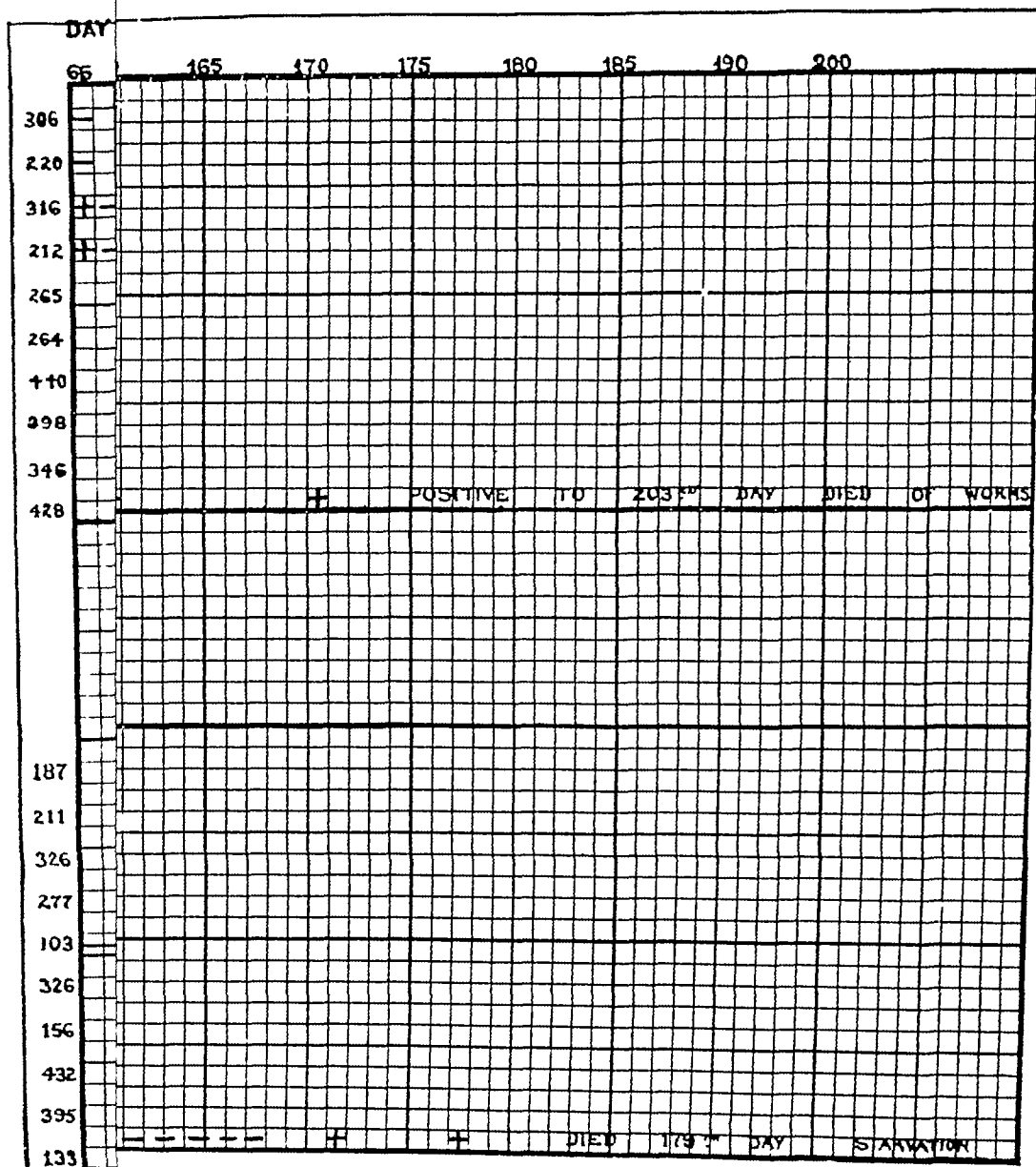
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IN a previous paper we gave an account of a number of medicinal plants which were known to the pharmacopœias and which grew in the Himalayas. We carried out qualitative as well as quantitative estimations of the active principles of a number of these plants in order to see if they can be employed for therapeutic purposes. In some cases, we actually tested their value on patients and showed that these plants growing wild and in great abundance in these mountains could be substituted for the imported and often expensive products. Besides these, a large number of plants grow in these mountains which though not exactly the same as those actually used in the pharmacopœias, have similar properties active principles and actions and would form excellent substitutes. That such plants exist, is well-known but no effort had been made to work out their chemical action or medicinal properties. In this paper we propose to deal with a number of these drugs which we have investigated during the last few years. We have great pleasure in expressing our gratitude to Mr Wright, the Chief Conservator of Forests, Kashmir State and Mr Kaul, the Conservator of Forests, Utilization

Pigeon No.

Pigeon No.



faces are shown in Chart 4 None of the pigeons in Groups No 6 and 8

The alkaloid colchicine is liable to be affected by high temperature. The corms should, therefore, be collected early in the summer and dried at a temperature not exceeding 65°C. Attention to this direction may increase the percentage of the alkaloid.

It would appear from the above analysis that both the corms and the seeds of *C. luteum* or 'surinjan-i-talkh' sold in the Indian market could be used for therapeutic purposes in place of *C. autumnale*.

## (2) *Mentha arvensis* N O Labiata vern Pudina (Hind, Beng)

A number of species of mentha grow in India. *Mentha viridis* (spearmint), *M. piperita* and *M. incana* (peppermint), *M. sativa* and *M. aquatica* occur as garden plants. *M. arvensis* grows most abundantly in the western Himalayas in a state of nature.

The drug was well-known to the Greeks and Romans and was used not only for flavouring food, but also for medicinal purposes. Although many species of this plant grow in India, the Hindu physicians do not appear to have used it in their medicines.

*M. arvensis* is now used as domestic remedy in India on account of its stimulant and carminative properties. The amount of essential oil obtained from *M. arvensis* by steam distillation of the whole dried plant sent from Kashmir was 0.18 to 0.2 per cent. As the quantity we obtained was small, sufficient amount of oil could not be distilled to study its physical and chemical properties. The oil has the same odour, taste, and other characters as the peppermint oil used in the British Pharmacopœia and crystals of menthol can be easily obtained from it on keeping for some time. It is probable the fresh herb might give a better yield of the essential oil. As most of the peppermint oil used in this country is imported from Europe or Japan, it may be worth while to take advantage of the large natural resources and distill it for commercial purposes.

## (3) *Juniperus Communis* N O Coniferae It is known as Aarar in Hindi and in Arabic

Several species of Juniper are to be found on the western Himalayas from Kumaon westward. They do not appear to be much used in medicines locally, though the berries are sold in the bazaars by Mohammedan druggists. *J. communis* was known to the ancient Greeks, and they undoubtedly used it for its diuretic as well as its digestive properties. Although several varieties are known to grow in the Himalayas, the Hindu physicians do not appear to have made use of this plant in medicine.

We have examined two species of Juniper commonly growing in Kashmir namely, *J. communis* and *J. macrocarpa*. In general appearance, there is not much difference between them in their berries, excepting that the latter are somewhat longer in shape. The amount of volatile oil obtained by steam distillation was 0.25 per cent and 3.24 per cent respectively from *J. communis* and *J. macrocarpa*. We could not investigate the physical and chemical properties of the former as

The following points suggest that it is of the same nature —

- (a) It occurred on a diet deprived of the antineuritic fraction of vitamin B (Groups No 2 and No 3)
- (b) It occurred on a diet showing a marked deficiency of the antineuritic vitamin (Groups No 10 and No 11)
- (c) It did not occur on diets containing a sufficiency of vitamin B as shown by the absence of polyneuritis (Groups No 4, No 5 and No 12)
- (d) It had no relationship to a high starch intake and occurred on a minimal consumption of starch
- (e) Its presence was associated with prolongation of life, and to some degree, with maintenance of body weight
- (f) The absence of the condition or its disappearance at certain stages of experiment was usually followed by death
- (g) The chemical examination of the faeces of the affected pigeons made at intervals showed a starch content varying from 38 per cent to 45 per cent
- (h) The starch resisted the action of amylase and ptyalin

While the condition of refection was observed by Fredericia on a diet deprived of both the antineuritic factor and vitamin B (*sensu strictu*), we have found it to occur on a diet deficient in the antineuritic fraction only. This agrees with the results obtained by Roscoe who observed the occurrence of refection on diets deprived of either factor and found that its presence both maintained normal growth and prevented the occurrence of neuritic symptoms.

#### *The transmission of Refection by feeding with Refected Faeces*

Fredericia and his collaborators have found that refection occurs spontaneously and is transmissible only in young rats, on the diets used by them, and have found that it did not occur in pigeons on a diet of polished rice with or without feeding with refection faeces. The numbers used in his pigeon experiments were very small. As we found the condition to occur spontaneously in a small proportion of adult pigeons out of batches of considerable size on a diet of autoclaved rice and autoclaved dhal, we have attempted transmission on this diet as well as on rice diet alone by feeding adult pigeons with refection faeces.

#### *Experiment No 2*

Four groups of ten pigeons each were put on the following diets

Group No 6	Autoclaved rice
Group No 7	Autoclaved rice plus autoclaved dhal
Group No 8	Autoclaved rice plus 0.5 grammes refection faeces daily.
Group No 9	Autoclaved rice, autoclaved dhal plus 0.5 grammes refection faeces daily

The occurrences in regard to these groups including the presence of starchy faeces are shown in Chart 4. None of the pigeons in Groups No 6 and 8

Name of species	Source	Total alkaloid per cent	Ephedrine per cent	REMARKS
<i>E. vulgaris</i> (stem & green twigs)	Not definitely known	0.92 to 1.49	0.5-0.65	Average of 6 samples
<i>E. vulgaris</i>	Kut range (Kashmere)	1.02	} 0.5 to 0.6	
<i>E. vulgaris</i>	Rampur on the right bank of the Jhelum at an altitude of 4750 ft	1.27		
<i>E. pachyclada</i> <sup>fil</sup> or <i>E. intermedia</i>	Simla, Hazara and Kashmir	1.80		
<i>E. pachyclada</i> or <i>E. intermedia</i> var <i>Tibetica</i>	Jhelum Valley	0.25 to 0.6	Mainly pseudo ephedrine	Average of 3 samples
<i>E. vulgaris</i> Chinese (Ma Huang)	China	0.95	0.56	Chen and Reid's analysis

The berries, roots, woody stocks and branches have been found to contain very little ephedrine. The green stems are the only parts which give the highest amount of the alkaloid. The collection of the drug in the autumn before the winter frost has set in is essential to a good yield of the alkaloid.

The above results were obtained from the best selected and carefully identified samples, but a number of species of ephedra are known in which the total alkaloids do not exceed 0.2 per cent of which ephedrine is sometimes less than 0.01 per cent. The different species are so closely allied in their botanical characters, that only a chemical analysis can show their value as a commercial article. There is every possibility of adulteration in the best specimens with the lowest grade without fear of detection. Ephedrine is a drug of great therapeutic value. If some sort of control is not exercised over the collection as well as the careful selection of the drug, Indian ephedra will have little chance of competing with the drug obtained from Chinese or other sources in the foreign market. In order to secure a foreign market the following suggestions might be of interest.

- (i) A careful botanical survey of the best available species should be made.
- (ii) Indiscriminate collection of the drug should be stopped, as far as possible, by the Forest Department. Collection of the green stems instead of the whole plant will both economize labour and increase the value of the drug.
- (iii) Extermination of the useless varieties, and supplanting them with better ones might be attempted.
- (iv) The conditions under which we can grow the best varieties, the season of collection of the drug and its proper storage should be studied.
- (5) *Citrullus colocynthis* N. O. *Cucurbitaceæ*. It is called *Indravaram* (Sans.), and 'Indrayan' in Hindi and Bengalee.





drug is extensively used in this country in the form of an extract which is as active as the extract imported from Europe

(6) *Berberis asiatica* N O *Berberideæ*

Several species of *Berberis* grow commonly in India. *Berberis lycium* grows in western Himalayas from Gharwal to Hazara, *Berberis asiatica* grows in the Himalayas and Bihar and is commonly sold in the bazaars. The stem is commonly known as 'Darhalad' and the extract is known as 'Rusot'. The plant usually grows at an altitude of 6,000 to 10,000 feet above the sea-level. The Greeks knew the value of the extract of this plant, and used it in medicine. The Arabian physicians have used the aqueous extract from very ancient times, and their method of extraction is the same as described by the Sanskrit writers, who used it largely in their medicine. The drug was commonly used in India in the treatment of malaria, and as a general tonic. It was prescribed in painful affections of the conjunctiva in combination with opium, as an external application it was applied to any inflammation and was taken internally in affections of the gastro-intestinal tract, e.g., diarrhoea, dysentery, gastric and duodenal ulcers, etc. So extensively was it used that it found its way into the western medicine. It has also been used in the treatment of oriental sore, as an external application.

The extract commonly sold in the market as *Rusot* is a dark brown sticky mass of the consistence of opium. It has a bitter and astringent taste, is readily soluble in water and is partly dissolved in rectified spirit.

*B. asiatica* contains two alkaloids, i.e., berberine and oxycanthine, the root containing the largest amounts. Estimation of the alkaloidal content of specimens sent to us gave the following results —

	Total alkaloids	Berberine
Stem	1.95 per cent	1.29 per cent
Root	4.0 „	2.09 „

Berberine sulphate and hydrochloride are used as antipyretics and tonics, and are also used in menorrhagia. On account of its contracting action on the spleen it has been combined with quinine in the treatment of chronic malaria. From the high alkaloidal content of both the stem and the root, it would appear that *B. asiatica* would be an excellent source of berberine for commercial purposes.

(7) *Picrasma quassiodes* N O *Samarubæ* vern Kashmir

This is a small tree or a large bush generally found in the sub-tropical Himalayas and Kashmir. The bark and the leaves are used in the Punjab as a febrifuge, and as an insecticide. The general structure of the wood as well as the taste of *Picrasma quassiodes* closely resemble that of *Quassia excelsa* of the British Pharmacopœia and it has been recommended as a substitute for it. *Picrasma excelsa* is not found in India. As far as is known, Indian quassia is not yet a marketable drug.

So far no standard chemical method for the isolation of the active principle of this drug has been worked out. Quassin a crystallisable bitter substance

of loss of refecton and in the remainder it was persistent throughout. Observation was continued until the 150th day, all eight surviving and remaining persistently refected.

In the second group receiving cooked dhal (Group No 11) the occurrence of refecton was observed from the beginning. It was found to make its first appearance at intervals of from three to thirty-four days from the commencement of the experiment. Of the ten pigeons in this group which died from polyneuritis one showed only a short period of refecton and the other irregular periods. The remaining eight had continuous refecton from an early period and were alive and well on the 150th day.

In Group No 12 receiving cooked atta in addition to autoclaved rice all pigeons remained alive and well and showed no signs of refecton.

In this experiment we have found a much higher incidence of refecton than in any other of our groups, 80 per cent of the pigeons receiving cooked dhal showing continued refecton with survival. There are two factors which may be concerned in its occurrence to this large extent.

- (a) A suitability of the cooked dhal for its production
- (b) The influence of a degree of vitamin B deficiency rather than its complete absence

We have not made exact observations on the antineuritic vitamin content of the cooked dhal. The occurrence of some cases of polyneuritis on this diet shows its deficiency, but we would not expect the complete destruction of the antineuritic fraction from the process of cooking used. The survival in good health of such a large proportion of the pigeons under experiment, in a permanently refected state, would suggest that refecton in these adult pigeons is capable of supplementing the supply of vitamin B sufficiently to make up such defect as existed. The results are in marked contrast with what was found in the case of pigeons fed on a similar diet in which the antineuritic factor had been destroyed. As regards the influence of the dhal itself we have seen that in our experience refecton did not occur on a diet of autoclaved rice alone but appeared when autoclaved dhal or cooked dhal was added. Kon and Watchorn (1928) have shown that refecton occurs more readily with some starches than with others and that the protective action is reduced by heating. They have found potato starch in the raw state to produce refecton with great regularity in young rats. It would appear that the starch of dhal is favourable to its occurrence and more favourable when cooked than when submitted to the higher temperature involved in autoclaving at 120°C.

The average composition of the two kinds of dhal used was —

	Moisture	Protein as dry gluten	Fat	Carbo-hydrate	Ash	Fibre
Moong dhal	10.2	23.3	2.4	60.6	3.5	4.4
Arhar dhal	10.1	21.8	2.7	61.5	3.9	2.2

chiretta *S. chinensis* is said to be more bitter than the official variety, and has come into use extensively

(9) *Aconites*, *N O Ranunculaceæ* vern Bish Bachnab

Many varieties of aconite grow in India in the alpine and sub-alpine regions of the Himalayas from Nepal to Kashmir Altogether there are 24 specimens, some of them are extremely poisonous while others are quite harmless According to Watt, six species of aconite recognized by the botanists grow in India with 2 or 3 varieties under 2 or 3 species These are (1) *A heterophyllum* (2) *A napellus* Linn, (3) *A ferox*, (4) *A lycoctonum*, (5) *A luridum*, and (6) *A palmatum* Some of these are active, others are absolutely inert In the European market, however, all the Indian forms are classified as *A ferox*, and it thus comes about that the aconite of commerce is an indiscriminate mixture of all forms whether active or inactive To clear up this confusion Goris (1901) and Stapf (1905) classified the Indian aconites into 3 types according to their being annual, perennial, or biennial, and from the structure of the roots According to this new classification the position of the Indian aconites of commerce is as follows —

(1) *A heterophyllum* is non-toxic and is employed medicinally in this country as aphrodisiac and tonic

(2) True *A napellus* sold in the bazaars is the European variety imported into the country *A chasmanthum* (Stapf) was formerly mistaken for true *A napellus*

(3) The so-called aconite *ferox* of Indian commerce is a mixture of four species, most of it is *A demorrhizum* and *A balfouren* both containing pseudoaconitine, but small quantities of *A spicatum* and *A lancinatum* which contain the non-crystalline bih-aconitine, sometimes occur The true *A ferox* does grow in Nepal and it is rare species

(4) *A lycoctonum* and *A palmatum* are both non-toxic

The following table gives the results of our chemical assay of the common aconites met with in the bazaars of India

Chemical assay of *Aconites* met with in the Indian market

Name according to old classification	Name according to new classification of Stapf	Name of the alkaloid isolated	Percentage of the total ether soluble alkaloids
<i>Aconitum napellus</i> (Mohri) 1	<i>A chasmanthum</i> allied to European	Indaconitine	4.50
<i>Aconitum napellus</i> 2	Do do	Do	4.28
<i>Aconitum ferox</i>	This specimen was a mixture of <i>A. demorrhizum</i> and <i>A. balfouren</i>	Pseudoaconitine	0.86
<i>A heterophyllum</i>	Belongs to the anthera type of Stapf	Atisine	0.38
<i>A lycoctonum</i>	Belongs to the perennial type of Stapf and includes <i>A luridum</i>	Lycaconitine	Only a minute trace of the alkaloid was obtained



# THE COMPARATIVE ACTION OF EPHEDRINE AND PSEUDO-EPHEDRINE FROM INDIAN VARIETIES OF EPHEDRA, ON THE HEART

BY

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EPHEDRINE was isolated from the Chinese plant Ma-huang by Nagai in 1887 and its pharmacological action was at first studied by Amatsu and Kubota in 1917. Since that date considerable experimental work has been done on the action of the Chinese and European varieties of ephedra, by various workers like Chen, Schmidt, Meek, Chow, Read, Fujii and others. The Indian varieties were studied by the senior author (1928), who found that the physiological and chemical reactions of the Indian alkaloid were in the main, identical with the alkaloid obtained from the Chinese varieties. An attempt was then made to study the action of ephedrine and pseudo-ephedrine from the Indian varieties of ephedra in detail on some of the tissues of the body. The alkaloids were isolated by Dr S Ghosh and Mr A T Dutt, of the Department of Chemistry, from the Indian variety *Ephedra vulgaris*, Rich (also known as *E Gerardiana*, Watt, *E distachya* and *E monostachya*, Linn). They specially prepared, and purified the hydrochlorides of ephedrine, and pseudo-ephedrine, and these were used in all our experiments.

The consensus of opinion is that the pressor effects after intravenous injections of ephedrine are due to the vaso-constriction produced by stimulation of the sympathetic nerve endings and ganglion cells. Although the stimulant action on the sympathetics has been studied in detail, little attention has been paid to the action of this alkaloid on the vagal mechanism. Chen and Schmidt

is rice Dhal is used to a certain extent by those whose staple dietary may be either rice or atta It has frequently been added to a rice dietary on the assumption that it would be likely to be of value in the prevention of beri-beri It is regularly issued by forest firms in Burma to their employees in areas, where beri-beri is common, for this purpose, but in our experience its use does not appear to be of much value (Taylor 1928) We have no exact knowledge of the antineuritic value of dhal or atta in the cooked state and have accordingly carried out certain feeding experiments on pigeons with these two articles of diet cooked exactly as prepared for human consumption

A mixture of moong dhal and arhar dhal was cooked by slow boiling in a small quantity of water, boiling being continued until it formed a thick paste This took about half an hour For administration to pigeons the cooked dhal was made up into small pills The atta was prepared in the form of the ordinary *chapati*, a dough being made from the flour and a thin cake of it cooked rapidly on a hot metal plate with the help of a little ghee (clarified butter fat)

In Section 1 of this paper we have seen that in the case of the pigeons of Group No 4 the daily consumption of fresh dhal was approximately six grammes and that this, added to a diet of autoclaved rice, prevented the occurrence of polyneuritis and was capable of ensuring complete maintenance The cooked dhal and *chapati* were accordingly hand-fed to the experimental pigeons in this quantity, autoclaved rice being available to the limits of appetite

Three groups of ten pigeons each were used

Group No 10	Autoclaved rice plus six grammes cooked dhal	
Group No 11	Do	Do
Group No 12	Autoclaved rice plus six grammes cooked atta	

The occurrences in regard to these groups are shown in Chart 5 Observation was continued for 150 days, no further change taking place after the termination of the chart

All groups showed a loss in weight of about 12 per cent and the rice consumption was very similar to that of Group No 4 of the first experiment Chart 5 shows that of the 20 pigeons in Groups No 10 and 11, four pigeons died of polyneuritis One of these birds showed the typical appearances of beri-beri columbarum We therefore have definite evidence of deficiency of the antineuritic fraction of vitamin B which is attributable to the effect of cooking on the dhal, a similar quantity of the fresh dhal having previously been found to prevent the occurrence of polyneuritis

The presence of refection in the remaining pigeons is a complication of the experiment which prevents us from observing to what further extent polyneuritis might have occurred In contrast with the findings in Groups No 10 and No 11 we find no evidence of vitamin B deficiency in the pigeons of Group No 12 All survived and none showed refection

McCarrison (1928) has found that fresh dhal and atta are of approximately the same value in the feeding of young rats over a short period of observation,

fig (a). The causes of rise of the blood-pressure would, therefore, appear to be (1) stimulation of the sympathetic nerve terminals and (2) sympathetic ganglion cells, chiefly the former

The next question is why the stimulant action on the heart, is not apparent when the drug acts by stimulating the sympathetic. The action of ephedrine is not confined to the vaso-motor fibres of the sympathetic, but the drug also stimulates the accelerator fibres in the heart as shown by the fact, that if the heart is perfused with such high dilutions as 1 in 100,000, a marked augmentation of the force and frequency of the beat is observed. It would appear, therefore, that the accelerator effect in intact animals is counter-balanced by some other factor or factors brought simultaneously into play. We proceeded next to investigate this. In order to do so, we first studied the effect of the drug on the inhibitory mechanism of the heart. To find out whether the vagus centre in the medulla was affected by the drug, the animal was pithed through the foramen magnum. The brain including the cerebrum, cerebellum and the medulla was thus destroyed, and the action of the higher centres was thus removed. Ephedrine injections in such animals do not produce any materially different results from those observed when the higher centres were intact. Section of the vagi also does not alter the response of the auricle, ventricle or the blood-pressure after an injection of ephedrine. It may be concluded from this, that the possible site of stimulation is peripheral and not central.

A perusal of Graph I, fig (c) shows that injections of ephedrine in animals, in whom paralytic doses of nicotine were previously administered, produce a distinct increase in the amplitude of contractions and frequency of the beat of both the auricle and the ventricle. Graph II, fig (b) shows that the same phenomenon is observed after the vagus terminals are paralysed by atropine. It may be argued from these experiments that the peripheral inhibitory mechanism of the heart, i.e., the terminations of the vagi as well as the ganglia are stimulated by ephedrine.

Stimulation of the vagus, however, in the neck in decerebrated animals by tetanizing induced current, showed no marked variations in the strength of the current required to produce vagal inhibition of the heart, before and during the height of ephedrine action. Occasionally, the vagal tone was slightly improved, a lesser strength of the current being required to produce inhibition and slowing of the heart.

The irritability of the heart muscle was studied by stimulating the surface of the ventricle by induced tetanizing shocks from a DuBois-Raymond inductorium. The minimum effective stimulus was determined, and an injection of ephedrine was then given. At the height of action of the alkaloid, the stimulus was repeated. It was found that after 2 to 5 mg doses a stronger current was required to produce the effect. With larger doses such as 10 mg to 15 mg, the decrease in the irritability was more marked. We feel justified in concluding, that the irritability of the heart muscle is decreased by ephedrine.

6 It appears that in the cooked state, dhal will not be capable of furnishing a supply of vitamin B to replace adequately any deficiency in a rice dietary. Atta will be of the greatest value for this purpose.

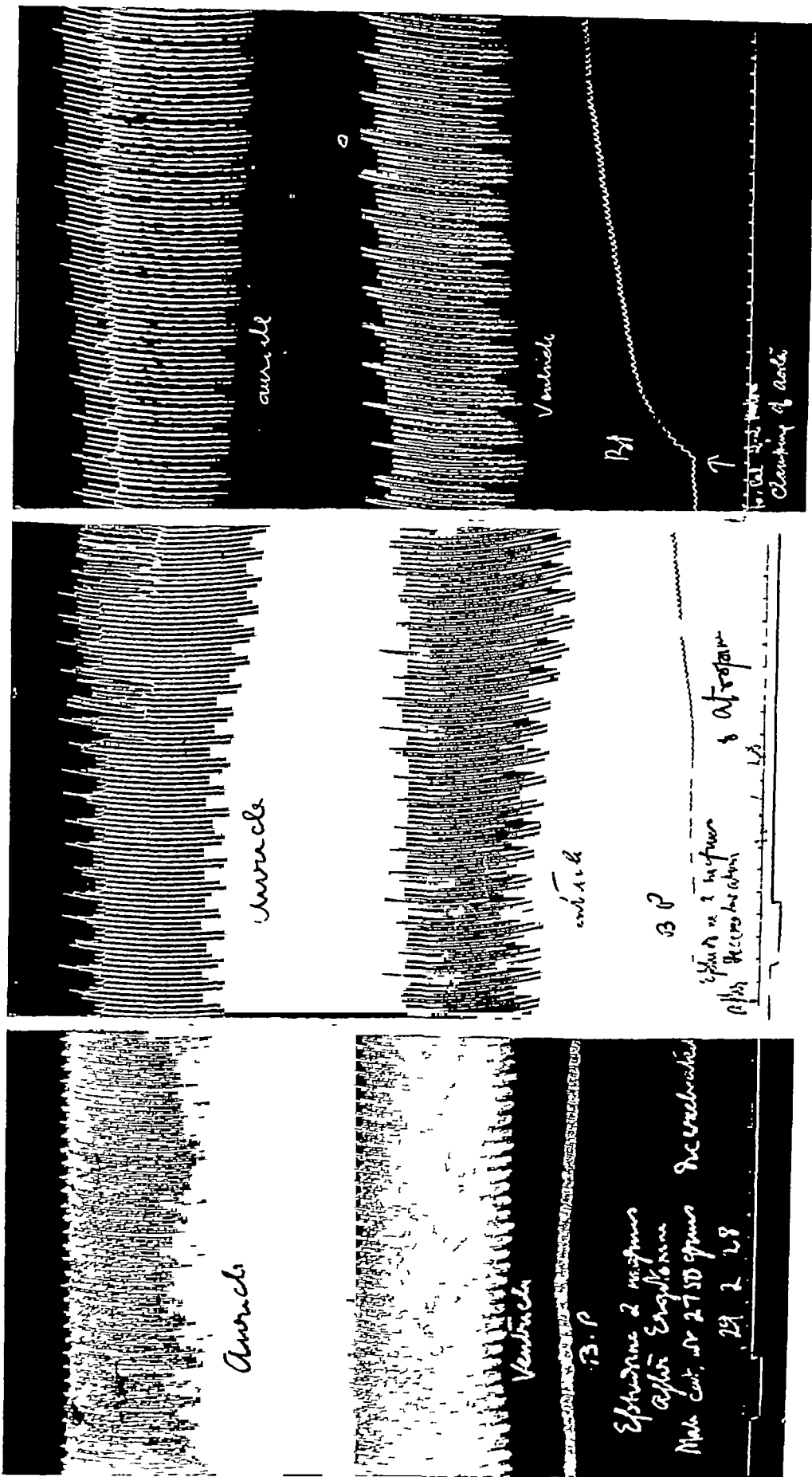
7 The deficiency of vitamin B in cooked dhal as contrasted with the sufficient content in cooked atta is probably attributable to the different methods of cooking used.

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# GRAPH II Blood-pressure and Myocardiogram records



It is significant that the appearance of these three dangerous carrier Anopheles, viz, *A. liston*, *A. maculatus* and *A. culicifacies*, immediately preceded and coincided with the season of greatest incidence of malaria, which, as we have shown in our last year's report (McCombie Young and Baily 1928) is in April and May

To our previous list of the Anopheline fauna of Coorg, *A. leucosphyrus* has now to be added

### *Preventive measures*

Having defined the Anopheline breeding grounds in Mercara, a detailed map of these was prepared and made over to the Commissioner of Coorg, Mr G W Priestley, I.C.S., and anti-larval measures were initiated

All the area in which the larvæ of these three carrier species had been collected, were sprayed with a larvicide of crude oil, obtained by the Commissioner from Madras, the operations being supervised by him personally, assisted by us

Two days after this spraying, we went over all the treated breeding grounds and we found that where to our knowledge larvæ had been numerous, they could no longer be collected, and it was evident that the operation had been effective

It was proposed to continue these operations, under Mr Priestley's supervision, and in view of the results which appear to be obtainable from such spraying, it would seem probable that a very considerable reduction in the output of carrier mosquitoes from domestic waters in Mercara should be effected

If these measures are continued, as was intended, until the onset of the monsoon rains, and also to some extent in the autumn months, the results, if successful, should be evident in a reduction in the spleen rate, and in the Mercara hospital attendance of town cases of fever

In our note of last year, we stressed the absence of any popular appreciation of the value of personal prophylaxis against malaria, and urged the need for the more general use of mosquito nets by the general population, pointing out that a useful net could be obtained for as little as some 3 to 4 rupees

In pursuance of this opinion, a sample of such a net was brought from Calcutta, and made over to a prominent Coorg lady who is interested in Red Cross work

### *Coffee Estates*

Last year we remarked on the difference in the malarial prevalence in the adjoining estates of Coovercolli and Dibidi, the spleen rate on the former being 38 per cent among the children and 33 per cent among adults and on the latter 100 per cent among the children and 73 per cent among adults, and we now endeavoured, in terms of anophelism, to ascertain something of the reason for the difference

The Coovercolli lines are situated on high ground, potential breeding grounds in their neighbourhood are few, and were not found to be prolific in Anopheline larvæ

In decerebrated animals an injection of 2 mg of pseudo-ephedrine produced practically the same action as in anæsthetized animals with intact brain. There was an increase in the amplitude of contractions of the auricles, and little or no effect on those of the ventricles, there was at the same time a prolonged rise of blood-pressure. Graph III, fig (b) clearly shows these effects. The blood-pressure was very low at the beginning of the experiment, and therefore the increase of blood-pressure was not sufficient to produce a dilation of the ventricle by offering more peripheral resistance. Decerebration and destruction of the cord do not interfere with this action of pseudo-ephedrine, showing that the action is not central.

Paralysis of the sympathetic ganglia also does not affect the action of pseudo-ephedrine. Graph III, fig (c) shows the effect of 2 mg of pseudo-ephedrine given in a cat, which had previously received sufficiently large doses of nicotine to paralyse the ganglion nerve cells. It will be observed that the amplitude of the auricles is markedly increased while the amplitude of the ventricles is increased only slightly. Here also, there is a slight dilation of the ventricles presumably due to the increased peripheral resistance. It would appear, therefore, that the sympathetic ganglia do not play an important part in producing the pressor and the accelerator effects of pseudo-ephedrine.

When this alkaloid is given intravenously to decerebrated animals whose sympathetics are paralysed by previous administration of ergotoxine, a rise in blood-pressure is still produced. There is at the same time, a definite stimulation of the amplitude of contraction of the auricles as well as of the ventricles. This is shown in Graph IV, fig (a). The rise in blood-pressure after the paralysis of the sympathetics might be due to two factors: increased cardiac output, or direct stimulation of the smooth muscles of the blood vessels, probably both these factors play a part.

The rise of blood-pressure in an anæsthetized, non-decerebrated animal without any stimulation of the ventricle suggests the stimulant action of the drug on the vaso-motor nerve endings. The persistence of the pressor effect, though seen to a lesser degree after the nerves are paralysed, suggests a direct stimulant action of the drug on the smooth muscles.

In spite of its stimulant action on the sympathetics, the effect of pseudo-ephedrine is not manifested on the heart in an anæsthetized or a decerebrated animal, as we found that there was only a slight increase in the amplitude of the auricles, and little or no effect on the ventricles. This is well illustrated in Graph III, figs (a) and (b). Section of the vagi also does not materially alter this action of pseudo-ephedrine. It appears, therefore, that the higher centres are not the seat of action of this drug. When the intracardiac ganglia of the vagus are paralysed by nicotine, an intravenous injection of pseudo-ephedrine shows practically the same effect as when the ganglia are intact as will be seen in Graph III, fig (c).

Graph IV, fig (b) shows that administration of the drug after paralysis of the vagus terminals by atropine, produces a marked stimulation of both the

of this fermenting fruit, added to water containing Anopheline larvæ in the laboratory, was in a couple of days lethal to them, although less so to Culicine larvæ similarly treated

If this isolated observation is correct, it would appear to be possible that this wild fig, which is used as a shade tree for coffee, may also have some value in discouraging Anopheline breeding

Two estate bungalows had recently been screened with gauze netting at a cost which the manager, Mr Nicolls, estimated at Rs 150 per bungalow, including labour. It was found by him that this screening does not interfere with ventilation or make the bungalow stuffy and that apart from its value in excluding mosquitoes, it gives immunity from the annoyance caused by other insect pests

The cost would not ordinarily be considered deterrent, and in view of the hyper-endemicity of malaria in Coorg, one would urge that such fittings should be found on every bungalow on coffee estates

Some figures are available which indicate the value of measures of hygiene among the labour force on this estate

Last year, the spleen rate among children was 100 per cent and among adults 91 per cent

This year, among 23 resident children, 10 had enlarged spleens or 43.5 per cent and among 16 resident adults, 6 had enlarged spleens or 37.5 per cent

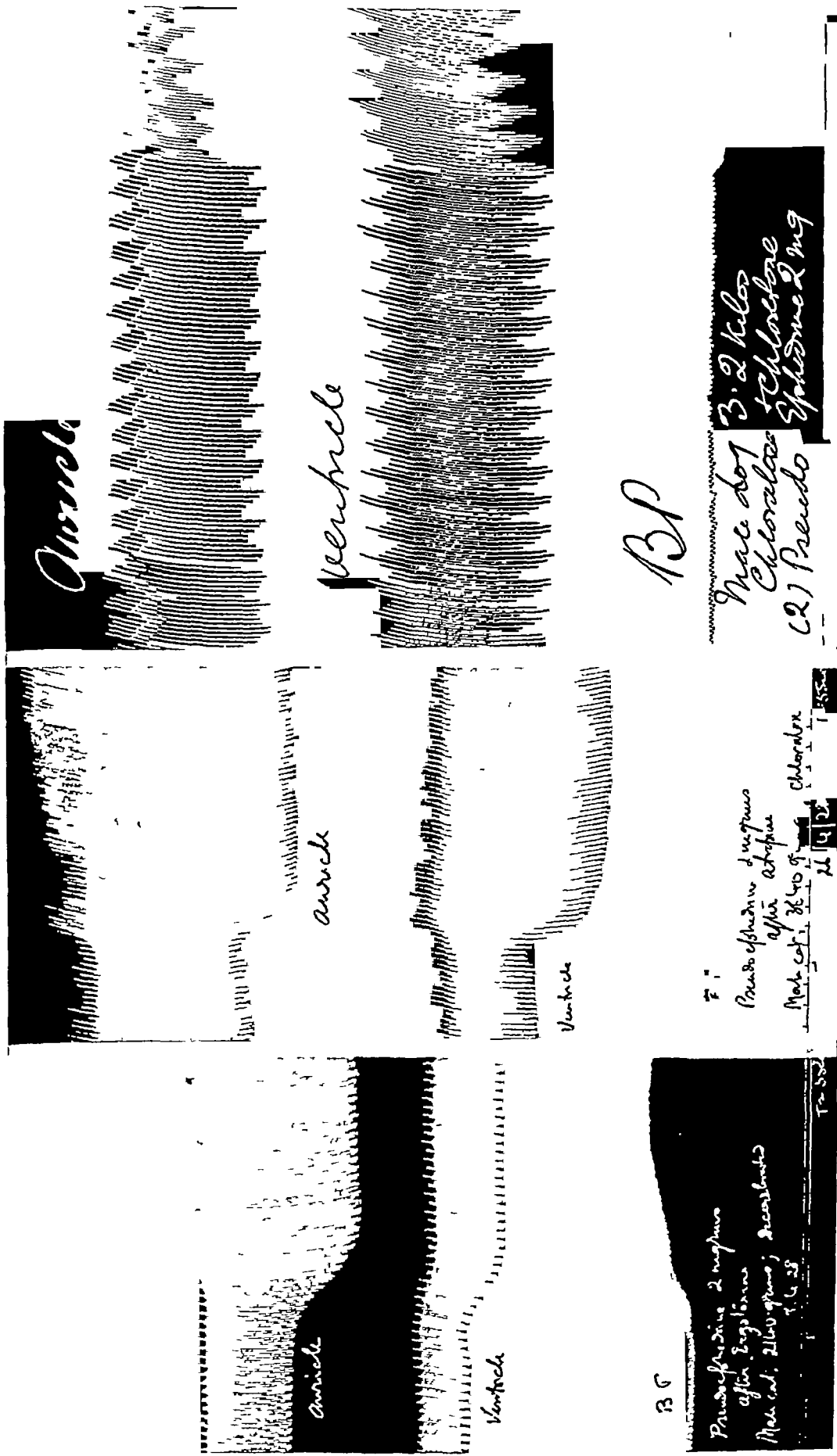
The number examined was small, as the resident labourers at that time of year are few, and the figure hardly warranted the calculation of a percentage, moreover, last year the observations were made at the termination of a malaria season, whereas this year's observations were made a month earlier, and owing to abnormal rainfall the malarial prevalence this year was likely to be low. With these reservations, the indications were that the health of the labour force had improved considerably as the result of the attention that had been given to it by the management

More general knowledge of the improvement in the health and efficiency of the labour force, which such expenditure of trouble and money can yield, should lead to an increase of attention to this aspect of estate management in the Coorg coffee industry, with beneficial results

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GRAPH IV  
Blood-pressure and Myocardiogram records



Note a marked increase in the force and frequency of the auricle and the ventricle when 2 mg of pseudo-ephedrine were administered to a decerebrated cat after paralysing the vaso motor sympathetic terminals with ergotamine. The rise in blood-pressure is still evident.

Note that 2 mg of pseudo-ephedrine given to a decerebrated cat whose vagal terminals were paralysed by atropine, produce a marked increase in the force and frequency of the auricle and the ventricle and a marked rise in blood-pressure

Note that a 2nd dose of 2 mg of pseudo-ephedrine produces auricular fibrillation in a dog

Division, who have given us every help in this research, and have supplied us with most of the specimens of these plants for our work

(1) *Colchicum luteum* N O Labacæ, vern 'Surinjan'

*Colchicum autumnale* which is official in the British Pharmacopœia does not grow in India, but several other varieties of colchicum grow in the western temperate Himalayas near Kashnir and Chamba. There are two species commonly sold in the Indian bazaars, one is sweet and the other bitter. The bitter variety is *C. luteum* which contains the alkaloid colchicine in fairly large quantity, the sweet variety also contains traces of an alkaloid which has been found to be physiologically inactive. *C. luteum* or 'Surinjan-i-talkh' is distinguished from the sweet variety 'Surinjan-i-shirin' by its bitter taste, smaller size, darker colour and a reticulated appearance of the corms.

The medicinal properties of this plant were well-known to the Arabs. The Kashmir Hermodactyls or 'Surinjan-i-talkh' is used by the Mohammedan physician as an alterative, aperient, especially in gout, rheumatism and diseases of the liver and spleen. In gout, it is combined with aloes, with ginger and pepper. It is used as an aphrodisiac, a paste is made with saffron and eggs and applied to rheumatic and other swellings, powdered root is sprinkled on wounds to promote cicatrization. 'Hiranya-tutha' or 'Haran-tutiya' a medicine of great repute in Afghanistan and Northern India, is a dark brown dry extract prepared mainly from the aqueous extract of *Colchicum luteum* and other species. In the Hindu medicine 'Tutham' or 'Tuttanjana' is the term applied to a collyrium made of copper sulphate and root of *C. luteum*.

The corms of *C. luteum* are occasionally adulterated with the corms of the sweet variety and another plant, viz., *Narcissus tazetta* belonging to the same natural order. This plant grows abundantly in Persia and is supposed to have similar properties. A variety known as *C. speciosum* (Stav.) commonly grows in Badghis and Khorasan and finds its way into India. The seeds of colchicum are not commonly sold in the Indian bazaars.

According to Dymock, Warden and Hooper (1893) the ether extract, i.e., the alkaloid-containing part was 1.31 per cent in bitter 'surinjan' obtained from Lahore and 0.69 per cent in sweet 'surinjan' (*Merendia persica*) from Persia. We have examined the corms of *C. luteum* and they appear to resemble *C. autumnale* in their general form. Chemical analysis shows that they contain a large amount of starch, small quantity of oily resinous matter, and a bitter alkaloid. Following the assay methods laid down in the United States Pharmacopœia we found the percentage of the alkaloid in the air-dried corms of *C. luteum* to be from 0.21 to 0.25, and in the seeds from 0.41 to 0.43. The alkaloid thus obtained has the same properties as that of the official alkaloid colchicine obtainable from the official *C. autumnale*. The U.S.P. requires 0.35 per cent of alkaloid in the corms and 0.45 per cent in the seeds. No standard, however, has been fixed by the B.P., it is merely recommended that the seeds should be employed for the preparation of the tincture and the corm for the extract or wine of colchicum.

Most of our experiments were performed on cats with pericardium slit open, and Barnard (1920) has pointed out that the right auriculo-ventricular valve rarely remains efficient when the pericardium is slit open. When the peripheral resistance rises, the ventricle is unable to empty its contents against such a resistance, and consequently dilates to a slight degree. The right auriculo-ventricular valve, therefore, does not remain competent and allows some blood to go back into the auricle during ventricular systole. The auricle thus becomes distended, and the stretched auricular muscle responds to the increased intra-auricular pressure by contracting more forcibly. It thus comes about that though there is an increase in the amplitude of contractions there is no change in the frequency of the heart beat. If the stimulation was produced through the agency of the accelerator mechanism, the force and frequency of the heart as a whole including the ventricles would have been accelerated. We are, therefore, justified in concluding that the stimulation of the auricles, is produced by distension of the auricle brought about by the mechanical factor already discussed. This is further supported by the fact that after the vaso-motor nerves are paralysed by ergotoxine, ephedrine produces no rise of blood-pressure and consequently there being no increase in the peripheral resistance, the increase in the amplitude of the auricle is absent. The direct depressant effect of the drug on the muscle fibres is manifested to a slight degree after ergotoxine.

When the inhibitory mechanism of the heart is put out of action by atropine or nicotine, the sympathetics have the full opportunity of manifesting their effect. The heart shows a well-marked stimulation in spite of the depressant action of the alkaloid on the myocardium.

The direct depressant effect of the drug on the myocardium of the heart is better seen when the injections are repeated. It has been shown by Chen and Schmidt that the first injection of ephedrine produces a well-marked pressor effect. After the second injection the rise of blood-pressure is very slight, while the subsequent injections hardly produce any pressor effects at all. That is the reason why the auricle does not show any stimulation after the second and subsequent injections, in fact it definitely shows signs of depression. This is clearly shown in Graph I, fig. (b).

What has been said above about ephedrine is true of pseudo-ephedrine also. The main difference between the two is that while ephedrine depresses the heart muscle, pseudo-ephedrine stimulates it. The stimulant effect of the drug on the muscle is, however, not so evident when there is high peripheral resistance. When this is no longer present, as is the case when the vaso-motor nerves are paralysed by ergotoxine, the drug can manifest its action fully, as will be shown by Graph IV, fig. (a). A marked stimulation of both the auricles and the ventricles, both as regards force and frequency of the beats is observed.

Although the paralytic effect of ergotoxine on the vaso-motor fibres of the sympathetic is well established, its paralysing effect on the accelerators of the heart is still doubtful. Evidence has been accumulating to show that ergotoxine does not paralyse the sympathetic nerve-endings of the heart. Otto (1928)

the amount of berries was not sufficiently large. The oil obtained from *J. macropoda* has the following characteristics —

	B. P.	<i>J. macropoda</i>
	Standard	
Optical rotation	$[\alpha]_{\text{D}}^{25} -3^{\circ} -15^{\circ}$	$[\alpha]_{\text{D}}^{25} -24.3^{\circ}$
Specific gravity	0.86 — 0.89	0.912

The colour, odour and solubility are the same as the official oil of Juniper. The nature of the oil is being further investigated as there is such a wide divergence in its physical properties.

- (4) *Ephedra vulgaris* N. O. Gentaceæ vern. Amsama Butshur, Cheva (Punjab), 'Mahuang' (Chinese)

Many samples of ephedra from Kashmir, and from Simla Hills have been analysed for the assay of the total alkaloidal content and the seasonal variations in the percentage of alkaloids therein. According to Watt (1890) three species of ephedra grow in India —

(i) *E. vulgaris* Rich. also known as *E. gerardiana*, or *E. distachya* and *E. monastachya* (Linn.) It grows abundantly in the drier regions of temperate and alpine Himalayas extending from Tibet to Sikkim ascending to an altitude of 16,000 feet. It grows abundantly in Shalai Hill north of Simla, and gives a very good yield of the alkaloid ephedrine.

(ii) *E. pachyclada* Boiss, also known as *E. intermedia*, Schrenk and Mey, is a tall shrub which grows in the dry stony regions of the western Himalayas and Tibet. A variety of *E. intermedia* known as *E. tibetica* grows abundantly in parts of Kashmir.

(iii) *E. peduncularis* Boiss, also called *E. alba*, *E. alata*, Meyer, is a tall scandent shrub growing in the stony grounds in Sindh, Punjab and Rajputana. No medicinal value is attached to it. Besides these, two other varieties grow in various parts of India, these being (1) *E. foliata* Boiss and (2) *E. fragilis*, but they are uncommon.

Two varieties of ephedra are at present known to grow abundantly in the mountain ranges bordering on the Jhelum River.

(i) *Ephedra intermedia* variety *tibetica*. Analysis of a number of samples of this variety showed a total alkaloidal content of 0.2 to 1.0 per cent of which 0.025 to 0.056 per cent was ephedrine and the balance was pseudo-ephedrine.

(ii) *E. vulgaris* or *E. gerardiana*. A number of specimens of this variety were examined and the total yield of alkaloids was found to vary from 0.8 to 1.4 per cent of which 0.5 to 0.6 per cent was ephedrine. This accounts for the fact that a number of specimens obtained from the same source have given such widely varying results so far as the ephedrine content is concerned.

Below is given the result of a number of analyses of ephedra conducted in this laboratory.



# THE MANGANESE IN FOOD-STUFFS

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THE part played by manganese in the animal organism and its necessity as a dietary element has recently been the subject of several investigations and as a preliminary to such experiments the determination of the manganese-content of food-stuffs is essential. This has been done for all the food-stuffs used in experiments on rats at the Deficiency Diseases Inquiry at Coonoor and various other common Indian foods. The object of this paper is to describe the method of analysis adopted and to record the results with such food-stuffs as have been examined.

The method of analysis is not new in main principles. As with all micro-methods though easy in theory it needs practice to make it work. In one particular, viz., the complete extraction of the manganese from an ash containing much silica, the method adopted appears to be new.

## METHOD OF ANALYSIS

*Principle*—The food-stuff is ashed and the ash dissolved in dilute nitric acid. The manganese is then oxidized to permanganate by ammonium persulphate using silver nitrate to catalyze the reaction. The amount of permanganate is determined by the depth of colour.

The chief difficulty in the process is getting the whole of the manganese into solution. If, as is often the case, the ash contains silica, this is insoluble in acid, and it adsorbs the manganese very tenaciously, so that it is impossible to extract the whole of the manganese from the ash. By fusing the ash with alkali carbonate the silica can be converted into alkali silicate which is soluble. If acid

Colocynth is widely distributed throughout India. It grows in a state of nature in the waste tracts of North-west, Central and South India. The fruit ripens in the cold season, and is offered for sale in Northern India by the herbalist in the months of December and January. The roots and the whole fruit (without the seeds) are commonly used in India, whereas only the pulp of the fruit is official in the British Pharmacopœia. The Indian varieties are nearly globular in shape and are usually of the size of an orange or smaller, when fresh, the pulp is spongy and juicy, but when dry the fruit becomes yellowish white, and contains a scanty yellowish pulp embedded inside the fruit. Peeled colocynth is not to be found in the market in India. The proportions of the pulp, seeds and rind are 15 62 23 respectively in 100 grm of the dried fruit. On an average, the fruit yields 12 to 15 per cent of dry pulp. All parts of the plant are very bitter and contain traces of an alkaloid, and the bitter principle colocynthine.

Colocynth is a very old remedy in Hindu medicine. The fruit is described as being cathartic, and useful in biliousness, constipation, fever and intestinal parasites. The root is used in ascites, jaundice, urinary disease and rheumatism. The Mohammedan physicians use this drug extensively in their practice, as a drastic purgative in ascites and jaundice, and in various uterine conditions especially against amenorrhœa.

There is practically no difference in the chemical composition between the Indian and European varieties. Both owe their physiological activity to an alkaloid and the bitter principle named colocynthine. The alkaloid is present only in a very minute quantity, and could not be isolated in a pure state. The following table gives the analytical result of the Indian colocynth which were analysed.

Pulp	Percentage of Extract
Petroleum ether extract	0 61
Sulphuric ether extract	3 17
Alcoholic extract (90 per cent)	10 90
Dry whole fruit (without seeds)	
Petroleum ether extract	1 36
Sulphuric ether extract	2 04
Alcoholic extract (90 per cent)	12 15

The bitter principle is nearly completely extracted by sulphuric ether after first removing the oily matter by petroleum ether. Traces of alkaloid can be found both in the ether and alcoholic extracts. Ethyl acetate is also a solvent for the bitter principle, and an extraction with this solvent after a preliminary treatment with petroleum ether, gives a residue of about 3 45 per cent on the weight of the dry pulp. The major portion of the bitter principle is soluble in water, is intensely bitter and gives a white precipitate with tannic acid, and from which it can be obtained in a purer condition. The average yield of the bitter principle may thus be calculated to be not less than 2 per cent on the weight of dry pulp which compares favourably with the drug used in the British Pharmacopœia. The

with dilute nitric acid and any insoluble matter filtered off through a small paper. The paper is then moistened with a solution of sodium and potassium carbonates (1.0 to 1.3) with a little potassium nitrate and dried and burnt on a platinum wire. The bead formed is then dissolved in the nitric acid extract. Silver nitrate solution and phosphoric acid are then added in the proportions of 2.4 ccs phosphoric acid and 10 ccs silver nitrate per 100 ccs of final bulk and the pink colour developed and read as before.

The standard permanganate may be prepared by any convenient method and that used in these experiments was made by weighing out potassium permanganate (A. R. quality) and checking against sodium oxalate. It is convenient to make a standard permanganate solution such that 1 cc = 1 mg of Mn, and to prepare the solution used for reading in the colorimeter from this. For reading solutions containing 1, 0.5, 0.2 and 0.1 mg per 100 ccs are convenient. In these solutions nitric acid, silver nitrate, phosphoric acid and ammonium persulphate are incorporated in the same proportions as in the unknown solutions to be tested, viz —

1 in 5 nitric acid	5 ccs
85 per cent phosphoric acid	2.4 ccs
1.5 gr/L silver nitrate	10 ccs
Water to	100 ccs

These dilute solutions keep well and should any doubt arise as to whether any of their permanganate has become reduced, their colour can always be restored to its original intensity by warming them and dropping in a few crystals of ammonium persulphate.

For comparing the colours of the standard and unknown permanganate solutions a Kober's colorimeter was used when the amount of permanganate was not less than 0.1 mg Mn per 100 ccs. With weaker solutions than this Nessler glasses were used. The colour of 0.005 mg Mn in 100 ccs is detectable and this amount of manganese when 5 grammes of food-stuff are taken corresponds to one part of manganese in one million of food-stuff.

*The quantities of reagents*—It is not necessary to be very exact in the quantities of the reagents added but the variation must not be large, or a brown tinge appears in the final pink solution and when this occurs, the method will give an erroneous result on the low side. The effect of varying the amount of the reagents were tried on a solution of a manganese salt of known strength and making up the final bulk in each experiment so that there were 50% Mn in a 100 ccs. A brown tinge and a low result was obtained —

- (1) By substituting 3 ccs or more of strong sulphuric acid for the 2.4 ccs of phosphoric acid. Indeed, when 10 ccs of strong sulphuric acid were used, no pink colour appeared at all.
- (2) By using 25 ccs or more of the silver nitrate solution in place of 10 ccs. If less than 10 ccs were used, e.g., 5 ccs, the colour was slower in appearing.

obtainable from the drug is supposed to be the active principle, but there are other bitters associated with it and there is also no accurate method of estimation of quassin. Following the method suggested for its isolation and comparing the result obtained with *P. excelsa* of the British Pharmacopœia, we obtained the following result —

	<i>P. quassiodes</i>	<i>P. excelsa</i>
Aqueous extract	8.36 per cent	5.04 per cent
Alcoholic extract	5.28 „	3.25 „
Bitter principle	0.31 „	0.48 „

The bitter principle was obtained by repeated treatment of the alcoholic extract with hot water, neutralising, concentrating the solution, and finally precipitating with tannic acid. The precipitate, thus obtained, was decomposed with freshly precipitated lead hydroxide, evaporated to dryness, and extracted with absolute alcohol. The alcoholic solution was evaporated in a water-bath and the residue weighed. White needle-shaped crystals were obtained mixed with other extractives and the residue was extremely bitter. The crystals which appeared in *P. excelsa* were much in excess than that of *P. quassiodes*. Besides the active principle quassin, the latter contains a bitter alkaloid to the extent of about 0.05 per cent and another fluorescing bitter substance soluble in chloroform amounting to 0.15 per cent.

(8) *Swertia Chirata* N. O. *Gentianaceæ* vern. Kirayat (Hind.), Bhunumba (Sans.), Chiretta (Beng.)

The herb grows abundantly in the temperate Himalayas from Kashmere to Bhutan and Khashia range. Most of the chiretta of commerce sold in the Indian bazaars comes from Nepal. A spurious kind of chiretta which is not bitter is met with which is said to be *S. angustifolia*, Ham. *Swertia decussata* is used in the Deccan as a bitter, and *S. corymbosa* and *S. pulchella* are much used as bitter tonics in the indigenous medicine in place of true chiretta. It has long been used by the Hindu physicians as a bitter tonic, stomachic, febrifuge and anthelmintic. An infusion of the drug is generally employed, but it forms part of many other preparations. The Mohammedan physicians also use it extensively.

The British Pharmacopœia does not lay down any definite standard for this drug. The common variety of chiretta as obtained from the Indian bazaar was assayed for the contents of its bitter principle by the method suggested by Zellner many years ago and which gives consistent results. Twenty grammes of the drug were extracted with boiling water containing a little calcium carbonate, till the last portion of the extract was devoid of bitterness. The solution was concentrated in vacuo, the residue is dissolved in hot 95 per cent alcohol and the alcohol removed and the residue taken up with hot water and filtered and the filtrate shaken up with ethyl acetate. The ethyl acetate was evaporated and the residue weighed. By this method the percentage of the bitter principle was found to vary from 1.42 to 1.52 and could be used in place of the official G. kurroo. Japanese

TABLE I—*concd*

	1	2	3	4	5
Expt No	Substance	Quantity taken	MANGANESE		Difference 4 — 3
			Added	Found	
7 8 9 10	Rice, polishings	5 grm	0 0 201 335	257 240 479 610	257 240 278 275
11 12 13 14	Rice, husk	5 grm	0 0 335 670	1 048 1,158 1,400 1,843	1,048 1,158 1,065 1,173
15 16	Rice, husk (Another sample)	5 grm	0 500	504 976	504 476
17 18	Milled rice (Sample II)	5 grm	0 50	48 98	48 48
19 20	Starch Sample I	5 grm	0 50	25 75	25 25
21 22 23 24	Wheat, starch	5 grm	0 0 0 50	22 23 25 70	22 23 25 20
25 26	Milk	20 c cs	0 50	18 65	18 15
27 28	Urine (normal)	100 c cs	0 100	0 103	0 — 3
29 30	Urine (jaundiced)	200 c cs	0 200	0 208	0 — 8

Chemical assay of these varieties shows that the alkaloid content of the so-called ferox form (*A demorrhizum* and *A balfourii* combined) is double that of the European variety of *A napellus* official in the pharmacopœia and of the Indian napellus variety, i.e., *A chasmanthum* is ten times as much

Biological assay of these roots shows that the ether soluble alkaloids (pseudoaconitine) of the so-called ferox form are 1.5 times stronger than aconitine obtained from the European variety of *A napellus* and the alkaloids obtained from the Indian variety of napellus (*A chasmanthum*) are 0.7 times weaker

The root of the so-called ferox form is, therefore, three times stronger than the European napellus root used in the pharmacopœia, and the root of the Indian napellus variety (*A chasmanthum*) is seven times stronger

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TABLE III

*Milligrams of manganese per kilogram of the various fractions on the basis of undried material*

Sample	I	II	III	IV	V
Paddy	118	49	47	38	32
Unmilled rice	30	20	22	20	20
Husk	221	140	115	88	52
First polishings	159	139	105	80	155
Second polishings	51	63	60	57	70
Milled rice	9	9	12	11	9

## RESULTS AND COMMENTS

The results of manganese determinations on various food-stuffs are given in Table IV. With very few exceptions, all the food-stuffs were found to contain manganese but the amount found varied very considerably both between various substances examined and between different samples of the same substance. Little or no manganese were found in any of the oils examined and none was found in cane-sugar, but with these exceptions, manganese is extraordinarily widespread in all classes of food-stuffs. It is impossible to devise a reasonable diet which is free from manganese. The manganese in cereal food-stuffs is present to a much larger extent in the outer layers than in the starchy part of the grains so that a large part of the manganese in such grains as wheat or rice is removed in the usual processes of preparing these for human consumption. In this way the amount of manganese in a diet is to some extent a measure of its sophistication. For example, the two diets proposed as cheap, sufficient and well balanced in Plimmer's *Food, Health, Vitamins* (1928, pp. 108 and 110) would give a manganese intake of 37 and 33 mg per man per day. If white flour were substituted for whole meal in these diets the manganese intake would drop to 5 and 10 mg per man per day, respectively. In this connection it is worth noting that tikitiki (item 68) (a preparation of rice polishings used for the prevention and cure of beri-beri) contains very little manganese (1.5 mg per kilo). Of all the cereals oatmeal (item 8) was the richest in manganese (348 mg per kilo)—the sample examined being a well known brand of tinned 'Scotch' oatmeal. The addition, therefore, of a daily plate of porridge from this oatmeal to a dietary would mean increasing the daily consumption of manganese by some 30 or 40 mg.

Teas from all the chief tea-growing districts in India were examined and one of these (from Katary in the Nilgiris) was the richest in manganese of all the substances examined. An experiment was made to ascertain how much of the manganese in tea passed into the infusion when the tea is 'made'. An

(1925) observed that the threshold electrical stimulation of the vagus, caused cardiac inhibition during the acceleration produced by ephedrine. Chen and Meek (1926) found, that ephedrine usually slowed the pulse rate in stimulating doses given intravenously, intramuscularly or orally in anæsthetized and non-anæsthetized animals, with intact vagi. Acceleration always takes place, when the vagi are paralysed with atropine. In larger doses, the rate is slowed irrespective of the vagal control.

In our series of experiments, the effect of the drug on the mammalian heart was studied in cats. The animals weighing between 2 to 3 kilos, were anæsthetized with chloralose or urethane supplemented with ether, when necessary. The blood-pressure was recorded in the usual way by a mercury manometer. For myocardiogram experiments, the chest was opened under artificial respiration, the pericardium was slit up, and the right auricle and ventricle were gently hooked up by fine curved pins and attached by fine threads to heart levers. The movements were recorded on a kymograph moving at a medium speed. For perfusion work, hearts of rabbits and kittens were used, and perfused with warm oxygenated Locke's solution, to which defibrinated blood was added. Langendorff's method was used for perfusion.

#### EPHEDRINE

Graph I, fig (a) shows the effects of an injection of 2 mg of ephedrine in a cat anæsthetized with urethane. There is the usual marked and persistent rise of blood-pressure, but the effect on the auricles, and the ventricles, is contrary to the findings of many investigators. The auricles in our experiments showed an increase in the amplitude of contractions, the ventricles showed a very slight decrease in the amplitude of individual contractions instead of the stimulant effect observed by many writers, there was no change in the frequency of the beats of either chamber of the heart. With larger doses, such as 5 mg, there was a decrease in the amplitude of both the auricles and the ventricles. [Graph I, fig (b)] The prolonged rise of blood-pressure, without any increase in amplitude of the ventricle, suggests an action on the vasomotor nerves. In order to determine the part played by the sympathetic ganglia in producing the rise of blood-pressure observed, we gave sufficiently large doses of nicotine to paralyse the ganglia. Ephedrine was then administered intravenously and Graph I, fig (c) shows the effect of such an injection. The pressor effect is still noticeable, but the increase, as compared with the condition when the ganglia were not paralysed is much less marked. The auricles and the ventricles are both stimulated, and it may be said, that the increase in the force and frequency of the heart was at least partly responsible for the rise in the blood-pressure. The paralysis of the sympathetic ganglia appears to have diminished the pressor response of the drug on the blood-pressure.

If vaso-motor sympathetic fibres are paralysed by administration of ergotoxine, the pressor effect is altogether abolished, and the effect on the auricles and the ventricles is one of very slight depression. This is clearly seen in Graph II,



TABLE IV—*contd*

Item No	Substance	Number of samples analysed	Highest	Lowest	Mean	
18	Cereals— <i>contd</i> <div> <div> Starch of potato  " " rice  " " wheat  Tapioca  Wheat, whole grains from Punjab  Wheat, atta (as purchased locally)  Wheat, American flour  " White bread  " chapatti (atta) </div> <div> } as prepared for market </div> </div>	1			2	
19		2	11	10	11	
20		4	5	4	5	
21		1			0.5	
22		1			43	
23		1		.	68	
24		1			12	
25		1			3	
26		2	53	30	42	
27	Oils <div> Cod-liver oil  Sesame oil  Linseed oil  Olive oil </div>	1			0	< 1
28		1			4	
29		1		..	15	
30		1			0	< 0.1
31	Condiments <div> Betel nut (prepared)  Betel leaves  Chillies (red, dry)  Pepper  Tamarind </div>	1			39	
32		1			14	
33		1			9	
34		1		.	64	
35		1			24	
36	Vegetables <div> Amaranth  Brinjal  Cabbage  Carrot  Cocoanut, fresh meat  Lettuce  Onion  Plantain (pulp)  Tomato </div>	1			5	
37		1			6	
38		1			1.3	
39		1			9	
40		1			15	
41		1			13.2	
42		1			33	
43		1			12	
44		1			31	

GRAPH I  
Blood-pressure and Myocardiogram records

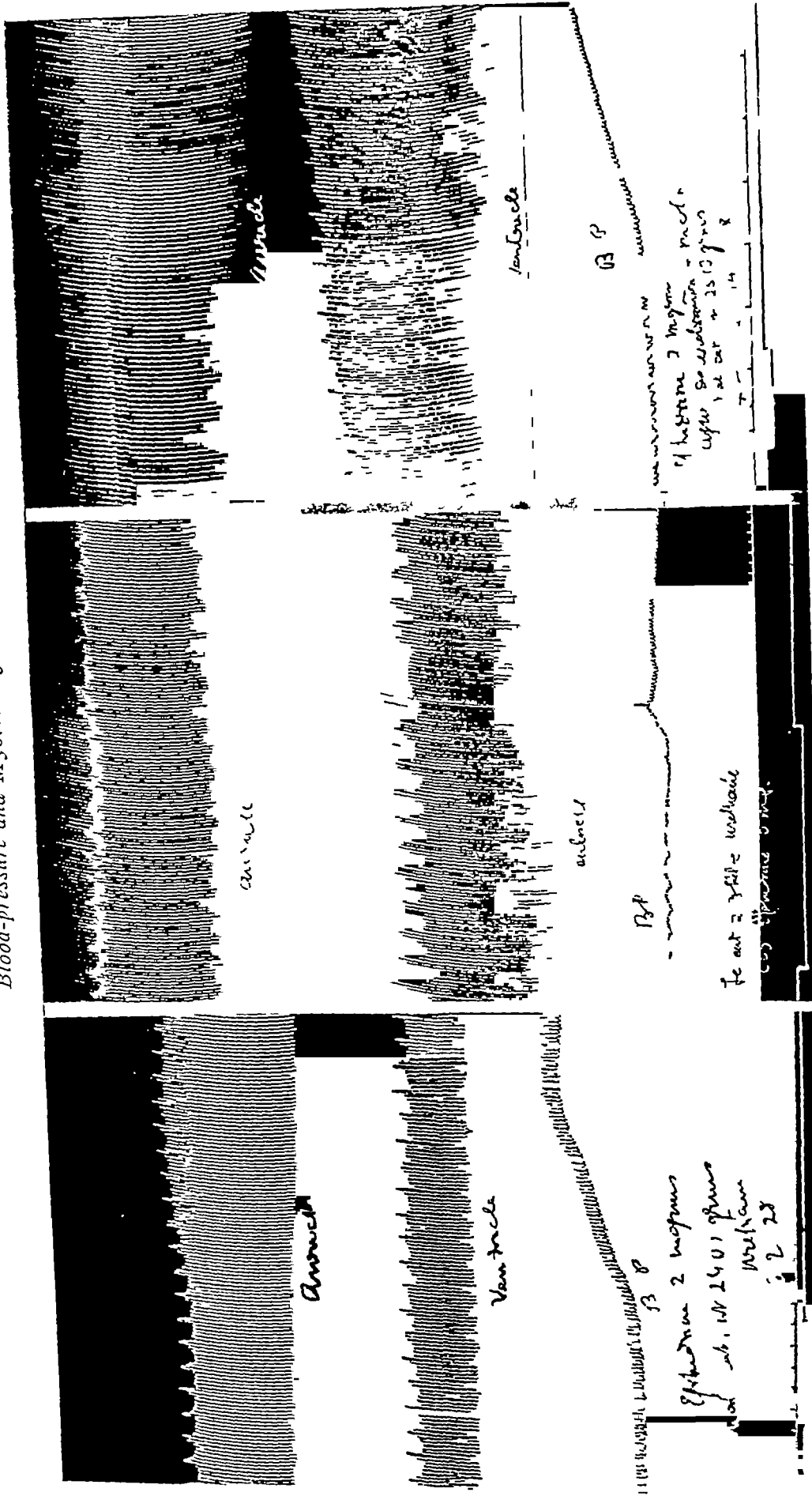


Fig (a)

2 mg of ephedrine given at the signal. Note increase in the amplitude of auricular contractions. The amplitude of ventricular contractions is slightly decreased. The blood-pressure shows a marked and persistent rise.

Fig (b)

Note the decrease in the amplitude of contractions of both the auricle and the ventricle when 5 mg of ephedrine were given after an initial dose of 2 mg.

Fig (c)

Note the increase in the force and the frequency of the beats of the auricle and the ventricle when 2 mg of ephedrine were given in a decelerated cat whose sympathetic ganglia were paralysed by nicotine. The rise in the blood-pressure is less marked than that in (a).

## SUMMARY

1 A method for the determination of manganese in food-stuffs is described

2 Manganese is widely distributed in food-stuffs Oils contain little or none, but all other classes of foods contain it in amounts which show very large variations both from one food-stuff to another and between different samples of the same food-stuff

3 The manganese in cereals like rice and wheat is largely lost in the processes of sophistication these often undergo in preparation for the market.

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The latent period was studied on frog's heart. A Stannius preparation was made by putting a clamp<sup>1</sup> over the crescent and the surface of the ventricle stimulated by single induced shocks. The point of stimulus was marked by a signal magnet writing exactly in the same perpendicular line. After taking normal curves, the clamp was removed, and an intra-hepatic injection of ephedrine was given. After allowing the heart to beat for a few minutes, the crescent was again clamped, and the ventricle stimulated as before. It was found that the latent period was not appreciably affected, even after large doses of ephedrine.

The refractory period was studied in the mammalian and the amphibian hearts, according to a modification of Waddell's technique. The writing lever was pulled away from the drum as soon as a circuit was made and a gap in the continuity of the tracing showed the point of stimulus. It was found that even after large doses of ephedrine such as 15 mg, the refractory phase is not in any way prolonged, the stimuli are still effective even at the beginning of the diastole.

Perfusion experiments show that ephedrine in such dilutions as 1 in 100,000, produces a marked increase in the force and frequency of the contractions of the mammalian heart.

It would appear, therefore, that the action of ephedrine consists in (1) stimulation of the vagus (2) stimulation of the sympathetics and (3) in larger doses depression of both the auricle and the ventricle by its direct action on the heart muscle.

#### PSEUDO-EPHEDRINE

The work done on pseudo-ephedrine by various investigators is comparatively less than that on ephedrine. Pseudo-ephedrine is an isomeride of ephedrine and is credited with having similar properties by Chen and Schmidt (1925), though its pressor effects are less marked. Fujii (1925) made a comparative study of pseudo-ephedrine and ephedrine, and came to the conclusion, that pseudo-ephedrine like ephedrine acts on the sympathetic nerve endings and ganglia, but unlike ephedrine, acts on the smooth muscles even in small doses. He also found that the rise of blood-pressure in rabbits following an intravenous injection of pseudo-ephedrine is less than that after ephedrine. Chen (1927) also noted the same phenomenon, and in one of his experiments he observed that the rise of blood-pressure produced by ephedrine was 3.2 times higher than that produced by pseudo-ephedrine.

We followed the same line of investigation to determine the action of pseudo-ephedrine, as we did in the case of ephedrine. Graph III, fig (a) shows the effect of 2 mg of pseudo-ephedrine given intravenously to a cat anaesthetized with urethane. There is a slight increase in the amplitude of the contractions of the auricles, mostly in the diastolic phase, the amplitude of the ventricles is hardly affected. There is, however, evidence of dilation of the ventricles as is shown by the fact that the lower curve has risen as a whole. The blood-pressure rose to a moderate degree and the rise was persistent.

slender and of lighter build than Europeans of a similar class. No data exist to show the normal relation of visceral weight to the total body weight in this community. In addition, in severe and advanced cases of sprue a condition of semi-starvation has existed over long periods of time, and it is well recognised that in starvation the fall of weight does not occur in equal proportion in all the viscera. Parsons (1924) utilizing Voit's data points out that in a starving animal 97 per cent of the adipose tissue may have disappeared and the store of the glycogen in the liver be so depleted as to have diminished that organ by half, yet the brain and the heart will not have lost more than 3 per cent of their original weight.

Daniels estimated that the average weight of the heart of Europeans in the tropics was 11 ozs and that of the liver 53 ozs. In five of our cases when the heart was weighed its mass equalled 12, 11½, 8, 4 and 2½ ozs, respectively, the average being 7.6 ozs.

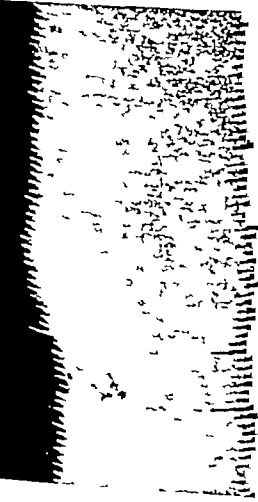
The last two hearts must be amongst the smallest met with in pathology, and suggest a specific atrophy of cardiac muscle out of all proportion to any decrease explicable on the basis of mere starvation. Manson-Bahr (1915) recorded similar findings in Ceylon when in one European case the heart weighed 3¾ ozs, while in that of a Burgher woman it was 6¼ ozs. The small heart often met with in this disease may have a special value in differentiating at autopsy between a case of sprue with hyperplasia of the bone-marrow and pernicious anæmia. In the latter condition not only is there an absence of general emaciation but actual hypertrophy and dilatation of the heart muscle is the rule. Thus Cabot (1926) records an actual increase in the weight of the heart in 22 out of 23 consecutive cases of pernicious anæmia.

Data concerning the weight of the liver also call for comment. In seven of our series this viscus weighed 27½, 30, 32, 42½, 46 and 55½ ozs, respectively, the average equalling 38.4 ozs.

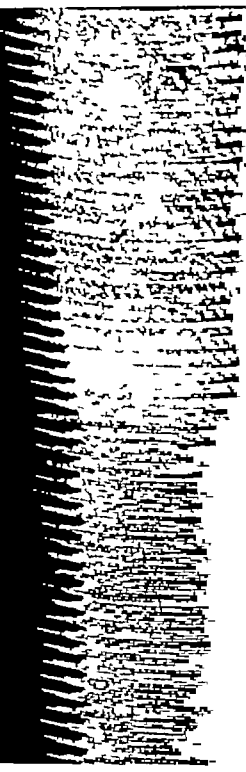
In our opinion that much stressed physical sign of sprue—decrease in the size of the liver—does not of necessity imply a specific atrophy of this organ but is rather related to chronic starvation in which depletion of the glycogen store consequent on deficient carbohydrate intake is an important factor.

### *General Features*

The body is emaciated often to an extreme degree and an almost complete absence of fat both under the skin and in the abdomen is the rule. Such traces of fat as may remain are generally of normal appearance and are not the lemon-yellow colour of pernicious anæmia. The skin is smooth or even glazed and pale, and may be ivory-white. A moderate amount of pigmentation was sometimes present on the face or abdomen but as most of our cases were Eurasians, the value of this finding was difficult to assess. Hæmorrhages, bruising or other vascular lesions were not noticed. Post-mortem changes were not evident until some hours after death owing perhaps to the desiccated and shrunken state of the body.



Auricle



Ventricle



Ventricle



Ventricle



Ventricle



B

2 mg pseudo-ephedrine  
at 2:10 p.m.

at 2:10 p.m.

at 2:10 p.m.

2 mg pseudo-ephedrine  
at 2:10 p.m.

at 2:10 p.m.

at 2:10 p.m.

Fig (a)

Note the slight increase in the amplitude of the contractions of the auricle, a slight dilatation of the ventricle and a moderate rise in blood-pressure after 2 mg of pseudo-ephedrine

Fig (b)

Note that an injection of 2 mg of pseudo-ephedrine in a decerebrated cat produces practically the same effect as in Fig (a)

Fig (c)

Note that after paralysing the sympathetic ganglia of a decerebrated cat with nicotine, in injection of 2 mg of pseudo-ephedrine results in a markedly increased amplitude of auricular contractions. The amplitude of contractions of the ventricle is very slightly increased. The rise in blood-pressure is still present

the naked eye The gall bladder generally contains bile in quantity and there is no obstruction to its flow into the duodenum

The spleen is dark, firm on section and of small size, averaging 4·2 ozs in weight Owing to its high incidence in Bombay, malaria must be excluded as an ætiological factor in the production of any pathological lesions observed in this organ at autopsy

The kidneys averaged 4·2 ozs in weight and generally presented no abnormality In two cases some increase in the interstitial tissue and reduction in the thickness of the cortex were observed, but these changes were not regarded as being necessarily related to sprue The supra-renal glands appear to be normal In only one instance were small opaque whitish lesions noted in the cortex

The abdominal lymph glands seem to be abnormally prominent, an effect which may be dependent on the disappearance of the mesenteric and retro-peritoneal fat The hæmolymp glands are also enlarged and appear hyperplastic

The pancreas is prominent, firm in consistency, but not fibrosed

#### *The Bone-Marrow*

It is generally stated that in the adult, the red marrow is normally found only at the ends of the long bones (Coloured Plates LVIII and LIX) Further, according to Pinney (1927), the red cellular marrow of the femur is confined exclusively to one small area at its upper end (diaphysis), whereas the whole length of the tibia is filled with yellow fat, all the red marrow having disappeared In our series the femur was examined in three instances and the tibia in five Considering the marked grade of anæmia stimulus to hyperplasia of the marrow elements existing in an aplastic or hypoplastic condition of the red marrow must be regarded as the commonest finding in sprue In two cases (Nos 3 and 6), however, section through the tibia showed marked erythroblastic reaction, the red marrow extending throughout the whole bone just as in pernicious anæmia, while in the femur of another case (No 4) a moderate grade hyperplasia of the red marrow was observed In the remaining cases (Nos 1, 5, 7, 8 and 9), the red marrow was either entirely absent (aplasia) or it occurred as a few islets of hæmatopoietic tissue surrounded by gelatinous yellowish coloured marrow (hypoplasia) The yellow fat normally present in the medullary canals of the long bones was found to be invariably replaced by a gelatinous material, greenish or light yellow in colour and sometimes resembling apple jelly in consistency There is evidence to show that the bone-marrow, under the stimulus of some hæmatopoietic toxin, passes through a hyperplastic stage, but that in the late and terminal phase the erythroblastic response fails with the production of the hypoplastic or aplastic condition of the marrow here described This is borne out by the blood picture of aplastic anæmia so often observed during life, as well as by the occasional appearance of a definite hæmolytic crisis which produces a sudden drop in both hæmoglobin and red blood corpuscles without signs of nucleated red cells in the peripheral blood These hæmatological features will be dealt with in more detail in a separate section of the enquiry

auricles and the ventricles, the amplitude of contraction being increased, and the force and frequency of the beat accelerated. The increase in amplitude, and acceleration of the rate are evidently due to stimulation of the sympathetics, which show a maximum effect, when the inhibitory mechanism is paralysed. It would appear, therefore, that simultaneously with the stimulation of the sympathetic or accelerator terminals, the vagal or the inhibitory terminals are also stimulated. The antagonistic effects of the two mechanisms are thus brought into play, and the net result is small on the contractions of the auricles and the ventricles.

Stimulation of the vagus in the neck by induced tetanizing current in decerebrated animals, does not show any appreciably marked change in the threshold stimulation at the height of pseudo-ephedrine action.

The excitability of the heart muscle was also tested as described previously in the case of ephedrine. It was found that pseudo-ephedrine increases the excitability of the heart slightly, a lesser strength of current being required to produce an effect at the height of alkaloid action after injection. This view is further supported by the fact that auricular fibrillations are sometimes produced after injections of even small doses of pseudo-ephedrine. Graph IV, fig (c) shows the effect of such an injection.

The latent period and refractory period were also studied by the same technique as applied to ephedrine. Pseudo-ephedrine does not alter the interval between the moment of stimulation and beginning of contraction, nor does it have any influence on the refractory period of the heart.

Experiments with perfusion of the isolated heart show that pseudo-ephedrine in even such high dilutions as 1 in 200,000 produces a well marked augmentation of amplitude of the contractions, and an increase in frequency of the heart beat even after ergotoxine and nicotine, pointing in all probability to its direct action on the heart muscle.

## DISCUSSION

From the experimental data given above, it will be seen that the effect of an injection of ephedrine will depend upon the sum total of its action on the sympathetic, the vagus and the myocardium. In a cat anaesthetized with urethane, the stimulation of the sympathetics will be counter-balanced by the simultaneous stimulation of the vagus. The direct effect of the drug on the musculature of the heart is only observed after larger doses such as 5 mg are repeated. With smaller doses such as 2 mg the increase in amplitude seen in the auricle is probably mechanical. Graph II, fig (c) shows the effect produced on the auricle and ventricle of a cat by artificially raising the blood-pressure by compressing the abdominal aorta. It will be seen that the blood-pressure rises and at the same time there is an increase in the amplitude of the auricles and a slight decrease in that of the ventricles. These are exactly the effects produced by an injection of ephedrine, the contraction of the arterioles produced by the drug here taking the place of artificial compression of the aorta.



Thinning and atrophy of the intestinal wall has been remarked upon by nearly every writer on the post-mortem appearance of sprue, and the changes to be observed in our sections support this contention

Whether this thinning and atrophy which affects the mucous membrane is the cause of the emaciation and malnutrition which characterizes the disease, or whether the atrophy of the intestines is part and parcel of the general starvation which results is a problem on which no definite opinion can be offered. We incline to the view that sprue is essentially a disease of the intestinal tract and that the atrophy of the epithelium is at least a contributory factor in the evolution of the disease. Bacterial invasion of the mucous and submucous coats is a common phenomenon quite apart from putrefactive influences, and it seems that chronic poisoning by such bacteria or their soluble products must play some part in the production of the clinical syndrome. These degenerative and atrophic changes are met with throughout the intestinal tract, but are most marked in the lower part of the small intestine.

Appearances suggest that the first change is noticeable in the villi, which show an infiltration with small round cells and a degeneration of their epithelial covering. Some congestion of the mucosa is present, but signs of active inflammation or of infiltration with polynuclear leucocytes is not seen. The process is essentially degenerative and non-inflammatory as we see it in terminal cases. The epithelial covering of the villi finally disappears, and the appearance of active vitality of the substance of the villus is lost. Eventually the villus appears shrunken and almost acellular, a condition which we describe as '*withering of the villi*'. The glands of Lieberkühn are little altered at first and indeed may remain healthy long after the villi have withered, but in many cases these too eventually become degenerated and granular, and show a loss of staining reaction, whilst the nuclei swell up and become disintegrated.

In some specimens slow atrophic dissolution of the whole mucous membrane results and the submucous coat is exposed by a process of erosion. This is not accompanied by any inflammatory reaction, or if such has occurred it leaves no traces in proof thereof.

The remains of blood pigment is frequently seen in the subvillous layers and this suggests the destruction of blood *in situ* such as might result from absorption of a hæmolytic poison like that excreted by *B. welchii* or other hæmolytic bacteria.

As has been stated in the remarks on the gross anatomy, ulceration has been absent in six cases and scars have never been seen. The submucous layer at certain stages shows congestion of the vessels and an increase in leucocytes, whilst in the late stages this layer shows an increase of connective tissue. The muscular tissue is certainly much reduced in volume, but the fibres and nuclei appear healthy. The serous coat is also normal and does not show the thickening which is usually associated with chronic inflammatory changes in the gut. The entire absence of fat in the intestinal wall contributes to the appearance of tenuity which is a naked eye as well as a microscopic feature.

showed that ergotoxine given in doses a hundredfold of those required to paralyse the vaso-motor fibres does not paralyse the accelerator fibres of the heart. There is every likelihood, therefore, that the stimulation of the heart might be due to stimulation of the cardiac muscles as well as of the sympathetic fibres. From the character of stimulation, i.e., increase both as regards the amplitude and the frequency of the beats, it would appear that both the muscular and nervous mechanisms play a part in producing the effect.

### SUMMARY AND CONCLUSIONS

(1) Ephedrine and pseudo-ephedrine obtained from the Indian varieties of *ephedra vulgaris* have a stimulant action on both the sympathetic and vagus nerve-endings of the heart. Ephedrine stimulates the sympathetic ganglia also.

(2) Both ephedrine and pseudo-ephedrine have a pressor action, the former being more powerful than the latter, the rise of the blood-pressure is produced by direct stimulation of the vaso-motor nerve-endings. The persistence of rise seen after paralysis of vasomotor nerves in the case of pseudo-ephedrine is due to increased cardiac output and also to direct stimulation of the smooth muscles of the blood vessels, causing them to contract.

(3) Pseudo-ephedrine stimulates the myocardium, while ephedrine in larger doses, e.g., 5 to 10 mg., depresses it.

(4) Stimulation of the ventricles observed by various investigators after injections of the alkaloids from Chinese and European varieties is not observed with the alkaloids obtained from the Indian varieties of *ephedra*.

(5) The physiological effect of increased peripheral resistance on the auricle of the heart when the pericardium is opened is discussed.

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*Visceral Pigment Deposits*

Fine granular pigment—sometimes golden or of darker hue—was constantly observed in the parenchyme cells of the liver, but not in the same amounts as in pernicious anæmia. The pigment was readily visible in hæmatoxylin eosin preparations and though some of it gave the Prussian blue reaction, it was often not brought into further prominence by treatment with acid ferrocyanide solutions. Hæmosiderin probably constitutes only a proportion of the pigment deposit in sprue, the nature of the remainder not having been determined. In one case at least its distribution was mainly around the hepatic vein, whereas in pernicious anæmia the storage of hæmosiderin chiefly occurs in the periphery of the lobules. Phagocytosed pigment was not observed in Kupffer's cells.

Iron pigment in limited quantity was also demonstrated in the kidneys, hæmolymph glands, intestinal walls and spleen. On three occasions a dark granular melanin-like\* pigment was present in the latter viscus, and once at least it was of malarious origin. It is interesting to note that while Fischer and Von Hecker (1922) describe hæmosiderosis of the liver, spleen and lymph glands in sprue, they maintain that the intestinal pigment is iron free and chemically allied to or identical with lipofuscin.

Much more work—both qualitative and quantitative—needs to be done on the pigment content of the tissues, but there can be little doubt that in sprue such deposits form a less characteristic micro-chemical feature than in pernicious anæmia. Nor is this a surprising finding for other data such as that obtained by the Van den Bergh test indicate that the hæmolysis though present is generally less intense in the former disease.

*Other Viscera*

The spleen showed some increase in interstitial tissue and thickening of the serous covering, as well as the presence of the pigments already described.

The kidneys were generally normal though hæmosiderin could be detected by special staining. In two instances there were slight interstitial lesions, but the secretory structures were normal except for the presence of such epithelial changes as may be attributed to early post-mortem modification.

The suprarenals (Plate LXIV, fig. 3) in several cases showed well marked vacuolation and breaking down of the cortical cells. These changes were often focal or at least limited to certain areas as if the result of some toxin acting locally. The appearances were suggestive of some agent acting during life and were certainly different from those observed as the result of early decomposition.

As we have already pointed out elsewhere (1926) that some features of the clinical picture of sprue, such as asthenia, pigmentation and low blood pressure, resemble those of Addison's disease, these microscopical findings have an additional

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\*Brown (1911) holds that the malarial pigment hæmozoin is not a melanin, but is identical with hæmatin. Its solubilities and micro-chemical reactions certainly support this view.

is added to this alkaline solution the silica is precipitated and one is no better off. If, however, the alkaline solution is sufficiently dilute and is poured straight into more than enough acid to neutralize it, the silicic acid is produced as a colloidal solution and although the colloidal particles doubtless adsorb the manganese, this adsorption does not interfere with the oxidation to permanganate nor with the reading of the pink colour. The colloidal solution thus formed appears perfectly clear but in a beam of light in a dark room shows a very fine Tyndall's phenomenon. It is quite stable and can be boiled and kept, for weeks if necessary, without flocculation.

*Details*—Five grammes or more of the food-stuff are weighed out into a dish or crucible of platinum, silica or nickel and ashed completely at a bright red heat. Care must be exercised in the early stages, until the organic matter is carbonized, to prevent spluttering. If the carbon does not all burn off easily, the partly ashed mass may be extracted with water filtered and the insoluble part with the filter paper ashed separately and added to the residue from the evaporation of the extractions. If chlorides are present to any appreciable extent, these are now got rid of by moistening the ash with strong sulphuric acid and heating again to dryness. A small trace of chlorides does not matter, for although an opalescence is produced when the silver nitrate (v 1) is added, this clears up when the oxidation to permanganate occurs. After ashing the process is varied according to the nature of the ash.

(I) If the ash is voluminous and insoluble in dilute nitric acid, it is now transferred to a nickel vessel (if not already in one) and fused with a mixture of potassium and sodium carbonate (1.3  $\text{K}_2\text{CO}_3$  to 1.0 of  $\text{Na}_2\text{CO}_3$ )—the amount used being sufficient to give a fused mass—and a little potassium nitrate added from time to time. The fusion mass is now extracted with 100 to 150 ccs water and the extract poured without filtration directly into a 500 ccs Erlenmeyer flask containing twice the quantity of dilute nitric acid required for neutralization. The extract is generally not quite clear. To the mixture are added, 6 ccs of 85 per cent phosphoric acid, 25 ccs of silver nitrate solution (1.5 gramme per litre) and water to make about 200 ccs and the mixture boiled vigorously. Solid ammonium persulphate is then added little by little, with care to avoid loss by frothing, the boiling being stopped during the additions. In a little time (from a few seconds up to 10 minutes) the whole solution goes pink and the turbidity clears up—or should do so—leaving a perfectly clear (though colloidal if silica was present in the ash) pink solution. This is brought to the boil once more, cooled, another pinch of ammonium persulphate added and made up to 250 ccs. Where only a small amount of manganese is present the final bulk may be made 100 ccs or 50 ccs by proportionately reducing the quantities of the reagents used. A small reduction in bulk can be made by boiling down. The amount of manganese is then determined by reading the pink colour against a standard permanganate solution.

(II) If there is but little ash or if it is almost completely soluble in dilute (1 in 5) nitric acid, the above process is modified as follows.—The ash is extracted

were scanty and the large majority were normoblasts. Considering the severity of the anaemia in these cases, the utter lack of response on the part of the hæmatopoietic tissue of the marrow of the long bones constituted perhaps the most striking feature observed at autopsy.

Finally, it may be added that in every case the organs were stained for monilia, but, with the unimportant exception referred to in describing the tongue lesions, yeasts were never found. This bears out our previously published views that *Monilia ashfordi* bears no primary ætiological relationship to this disease.

#### COMMENTARY

Comparatively little has been written on the morbid anatomy of sprue and many of the most quoted references such as those of Thin (1890), Wethered (1890), Faber (1904) and Justi (1913) have been based on an examination of one or two cases. Faber injected the abdominal cavity of his case with formalin immediately after death and found intestinal ulceration unassociated with atrophy of the gut wall.

On the basis of this observation he explained thinning of the gut wall as being due to distension with gas and atrophy of the mucous membrane as a post-mortem phenomenon dependent on loss of surface epithelium and destruction of villi. Justi in the main supported these conclusions. More recently Faber (1927) has reopened the subject when discussing intestinal atrophy in pernicious anaemia and maintains that when a similar technique is adopted (i.e., the immediate intra-peritoneal injection of formalin after death) the signs of intestinal atrophy are absent in this disease also.

The influence of the above mentioned factors is certainly a matter to be considered and we hope to get an opportunity of investigating the method at subsequent autopsies. Quite apart, however from the vexed question of thinning of the intestinal wall and its causation, we do not consider that the slow destruction of the epithelium of the mucosa described in our cases can be adequately explained either by meteorism or necrobiotic changes, or by a combination of both factors occurring under tropical conditions.

The most careful descriptions of the morbid anatomy and histology of sprue are to be found in the well-balanced and eminently sane writings of Manson-Bahr, (1915 and 1924) whose work should be read in the original. Amongst other things this author rightly points out that cases of sprue which usually come to autopsy represent the terminal condition of the disease, the chronic starvation accounts for many of the late appearances, and that the only hope of discovering the initial and underlying cause depends on studying materials derived from very early cases. He also regards sprue as being primarily a disease of the alimentary tract—a view which we thoroughly endorse.

In addition to intestinal atrophy and nutritional disturbances, we have been impressed with the increase in the hæmolytic bacteria of the intestinal flora and the invasion of the atrophic mucosa with cocci and other organisms. These factors, we believe, result in a state of chronic toxæmia which further increases the

- (3) By using 2.5 grammes or more of ammonium persulphate instead of the 0.25 grammes which was sufficient to develop the colour

Variations in the temperature of the solution when the ammonium persulphate was added appeared to alter only the rate at which the colour appeared and not its final intensity. For example, at 30°C the colour did not begin to appear till 5 minutes after adding the ammonium persulphate and did not reach its full intensity till some 15 minutes later. At 100°C the full intensity was reached at once.

*The readings in the colorimeter*—When the two solutions to be compared were considerably different in colour, there was a tendency for the stronger solution to read too strong or what is the same thing for the weaker solution to read too weak. This can be corrected for in a similar way to that suggested for creatinine estimations (Newcomb, 1924) but with solutions which do not differ by more than 1.2 in manganese-content the error on this score is negligible in comparison with the errors in the rest of the analysis.

*Controls*—The method was controlled by —

- (1) Testing the reagents and apparatus used for manganese. This was done by blank experiments in which distilled water was substituted for the food-stuff and such experiments always gave no indication of manganese. Such an experiment was always made when a fresh supply of any reagent was introduced. Determinations in which no manganese was found (e.g., cane-sugar) also serve as controls of the purity of the reagents.
- (2) Adding known amounts of manganese (in the form of potassium permanganate or of a manganese salt) to a weighed quantity of some food-stuff and calculating the extra manganese recovered. The results are shown in Table I.
- (3) The consistency of duplicate (or triplicate) analyses. The results (other than those included in Table I) are shown in Table II.

TABLE I

*Showing the recovery of added manganese*

The figures for manganese are thousandths of a milligram

	1	2	3	4	5
Expt. No	Substance	Quantity taken	MANGANESE.		Difference. 4 — 3
			Added	Found	
1	Milled rice Sample I	5 grm	0	48	48
2			0	48	48
3			0	39	39
4			50	98	48
5			67	104	37
6			201	242	41

though in two instances there was extensive hyperplasia similar to that seen in pernicious anæmia. A moderate grade of hyperplasia was seen in another case. Such findings suggest that in sprue there is a toxin which primarily stimulates and later leads to the exhaustion of the hæmopoietic function so that in the terminal stages of the disease an almost complete aplasia results. This supposition is borne out by the condition of the blood as seen during life.

7 These and other studies lead us to believe that sprue is primarily a disease of the intestinal tract which, if progressive, results ultimately in degeneration and destruction of the absorbing and secretory tissues and the production of a condition of slow progressive starvation. The absorption of toxins from the damaged mucosa perhaps associated with actual bacterial invasion, appears to be an important factor in the progressive anæmia and other late manifestations of the disease.

#### ACKNOWLEDGMENTS

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We also wish to thank Lieut.-Col. I. Davenport Jones, I.M.S., and Major P. K. Gilroy, I.M.S., and their assistants on the staff of St. George's Hospital for kindly giving us assistance in the performance of autopsies on their cases at that hospital.

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TABLE II

Showing the consistency obtained in duplicate analyses (additional to the figures in Table I)

Five grammes of the food-stuff were taken for each determination

Expt No	Substance.	MANGANESE FOUND	
		$\gamma$	mg/kg
31 32 33	Unmilled rice	111 102 112	24 20 22
34 35	Milled rice Sample III	44 44	9 9
36 37	Milled rice Sample IV	50 48	10 10
38 39	Milled rice Sample V	50 52	10 10
40 41	Starch Sample II	51 56	10 11

#### THE DISTRIBUTION OF MANGANESE IN RICE

Rice, being the staple food-stuff of the south of India, was chosen for a careful examination of its manganese-content and for an investigation into the distribution of the manganese in the different parts of the rice grains. Five paddys\* were chosen all grown in south India, the names being—

- I 'Nellore' paddy
- II 'Kuruvar' grown 4 miles from Kumbakonam
- III 'Sirumani' grown 10 miles from Kumbakonam
- IV 'Madras Samba' at Kumbakonam
- V 'White Kuruvar' grown 7 miles from Kumbakonam

The distribution of the manganese in the various parts\* of these paddys is shown in Table III

\* The term 'paddy' is used to mean the whole rice including the husk. The terms 'first polishings,' 'second polishings' refer to the parts removed, after the husk has been taken off, by the two poundings in a mortar that rice is usually subject to



## POST-MORTEM RECORDS IN DETAIL

**Case 1** I L, Anglo-Indian Age 39

Some five weeks previously the patient was confined in St George's Hospital. The baby was premature (7 months) and died. She left hospital against medical advice, but the uterus was involuting satisfactorily at this time.

On the 25th January, 1924, the patient was re-admitted in a very anæmic condition with a history of diarrhœa—several stools being passed daily. Some mucus was present. The urine was normal. The temperature was only elevated on the day of admission and prior to death—otherwise it was subnormal. The condition was regarded as definitely one of sprue complicating childbirth. The patient became moribund and died on the 3rd February, 1924, but unfortunately neither records of the blood condition nor detailed clinical notes are available.

## AUTOPSY

A thin, small woman with multiple scars on the forearm and one scar above the right knee. The skin was loose and wrinkled but there was no jaundice. The muscles appeared normal, in the subcutaneous tissue there was a small amount of very yellow fat.

*A Thorax*

*Heart*—There was some serous slightly blood-stained fluid in the pericardium. The right heart was dilated and its muscle was flabby and of a yellowish, fatty appearance. Valvular disease was absent.

*Aorta*—No atheroma was present, but some linear fatty markings of the intima were noted. The intima of the pulmonary artery and the endocardium itself were pinkish red in hue.

*Lungs*—There was some tendency to emphysema at the edges of the lungs, while hypostatic congestion and terminal œdema were noted at the bases. Some chronic adhesions were present over the left and right lungs anteriorly, while the base of the left lung was adherent to the diaphragm.

*B Abdomen*

The mucous membrane of the œsophagus appeared pale and somewhat thin, while its vessels were injected.

The stomach was of normal size, some pinkish areas about the size of sixpence were present (? post-mortem changes).

The duodenum presented some injected vessels and petechial hæmorrhages in the mucosa, while the small intestine appeared thinned in places but nowhere was it ballooned with gas. Its vessels also were injected. The mesenteric lymph glands were not enlarged, while the large bowel appeared normal.

The liver was of small size actually weighing 30 ozs. The capsule showed dilated injected venules with thickening of its upper anterior surface. On section it was nutmeg in appearance, areas of reddish congestion alternating with zones of surrounding pallor. Its substance generally was pale and fatty. The gall bladder contained a small quantity of golden yellow thin bile, and no obstruction of the duct existed. Its mucous membrane showed chronic proliferative cholecystitis.

The spleen weighed 3½ ozs. Its capsule was wrinkled but no evidence of perisplenitis was observed. On section the fibrous tissue trabeculae were prominent, but in consistency the organ was soft. An old organized thrombus (? infarct) was present.

The pancreas appeared large and weighed 3 ozs. It was lobulated in appearance and firm in consistency. Post-mortem clot filled the pancreatic vein. Attached to the tail of this organ was an accessory spleen.

The supra-renals were normal.

infusion was made by pouring 300 ccs of boiling water on to 5 grammes of a Darjeeling tea (containing 192 mg manganese per kilo), letting it stand 5 minutes and then straining it off. Of the 0.960 mg of manganese in the 5 grammes of tea, 0.193 mg (20 per cent) were found in the 300 ccs of infusion ( $\approx 0.64$  mg per litre of infusion).

A selection of food-stuffs has been analysed sufficient to show that any ordinary dietary must contain some manganese and that many dietaries contain a considerable amount and that the variations in manganese intake on different diets are very large. It would be very extraordinary if this constant intake of manganese, which must occur in all animals, were without any effect on their metabolism.

TABLE IV

*Showing the manganese-content in milligrams per kilogram (parts per million) of the food-stuffs analysed on the basis of undried material*

Item No	Substance.	Number of samples analysed	Highest	Lowest	Mean	
1	Arrowroot	1			1	
2	Barley	1			10	
3	Cholam ..	3	205	19	82	
4	Cornflour	1			1	
5	Dhal	1			26	
6	Gram, sprouted	1			19	
7	Kambu	2	115	24	46	
8	Oatmeal, tinned	1			348	
9	Ragi	3	281	95	177	Standard deviations
10	Rice, polished	17	12	7	9.5	
11	„ unpolished	19	36	5	17	12
12	„ paddy	5	118	32	57	7.5
13	„ husk	6	221	52	130	
14	„ 1st polishings	5	159	80	128	
15	„ 2nd polishings	5	70	51	60	
16	Sago	2	7	3	5	
17	Starch of unknown varieties	2	5	5	5	

*B Abdomen*

The liver was not weighed but it did not present stigmata of atrophy such as are generally described as characteristic of sprue. The gall bladder contains much bile.

The small intestine showed no evidence of erosions or ulceration, but the lower 12 inches of the ileum was very congested and small petechial hæmorrhages were present on the summits of the mucosal folds.

Neither the stomach nor the small intestine presented the atrophic thinning described in sprue. The œsophagus was normal though the mucosa was pallid.

The tongue was large, pale and smooth, the whole mucous membrane appearing atrophied. Villi were not evident.

The large intestine showed congestion and redness of the mucous membrane but no hæmorrhages, erosions or ulceration were noted.

Some of the mesenteric glands appeared somewhat swollen and enlarged, but neither caseation nor other pathological changes were observed. The lacteals were defined but not abnormally so.

The pancreas was prominent and hard to the touch, but definite pathological changes were not ascertained.

The spleen and kidneys presented no abnormality.

*C Bone-Marrow*

Only the ribs were examined. There the red marrow was deposited through the cancellous tissue in normal quantity. Hyperactivity rather than the reverse characterized the red marrow in this anatomical situation.

## SUMMARY

From the clinical history of this case there can be no doubt that the patient suffered from sprue. Yet the findings at autopsy were confined to atrophic changes in the tongue and evidence of some irritative condition of the ileo-colon as indicated by congestion and the presence of petechial hæmorrhages. Unfortunately, the condition of the bone-marrow in the long bones was not investigated.

**Case 3** J. M.—English. Age 62. Male.

As far as we could ascertain this case was first admitted to St. George's Hospital with an afebrile diarrhœa on the 6th August, 1923. He gave a history of diarrhœa for the past six months without blood or mucus, the bowels acting 3 to 6 times daily. At this time the stools were loose, small in quantity and not offensive.

Blood smears showed B. T. parasites. He was treated with quinine, and also received 6 injections of emetine.

He was re-admitted to hospital on 19th October, 1923, with diarrhœa and pain in the back and again given emetine, but on 9th November, 1923, he left against medical advice.

He was re-admitted to hospital on 20th December, 1923, with sprue diarrhœa being placed on sprue diet and was discharged on the 4th January, 1924.

He was re-admitted on the 12th April, 1924, with afebrile diarrhœa. This constituted about the 6th attack. The tongue was glazed and sore at the right edge, while sprue stools were being passed, large in quantity and pale in colour but not frothy.

The blood was negative for malaria.

R B Cs	= 4,000,000 per c.mm.
Hæmoglobin	= 70 per cent
C I	= 0.9
Leucocytes	= 6,250 per c.mm.

TABLE IV—*concl'd*

Item No	Substance	Number of samples analysed	Highest.	Lowest	Mean	
45	Cane-sugar	1		..	0	< 0.4
46	Casein	1	.	.	13	
47	Coffee (Nilgiris)	1		.	30	
48	„ infusion	1			0.3	
49	Eggs, yolk	2	3	2	2.5	
50	„ white	2	0.6	0.5	0.6	
51	„ shell	2	20	15	17	
	„ yolk and white together	2	1.4	1.0	1.2	
52	Fish, sea	1	..	.	3	
53	Ginger	1			38	
54	Linseed meal	1		..	168	
55	Marmite	1		.	7	
56	Meat residue	1			4	
57	Milk	2	1	1	1	
58	Anamallais	2	119	110	115	Standard deviation
59	Assam	1	.		370	
60	Cachar	1			546	
61	Choonabatti	1			208	
62	Darjeeling	2	199	192	196	
63	Duars	1		..	227	
64	Nilgiris	4	736	234	415	
65	Sylhet	1			343	
66	Tera	1			283	
	Mean of all dry Teas	14			304	163
67	Tea, infusion	1			0.6	
68	Tiki-tiki	1			1.5	
69	Yeast, dried	1			11	

The heart weighed 11½ ozs. The cardiac muscle was firm, in good condition and presented no stigmata of degeneration. The valves were not diseased but there was some stiffening around the orifices of the coronary arteries, and atheromatous patches were noted in the aorta. Atheroma was absent from the pulmonary arteries.

### B Abdomen

Where the coils of intestine were unsupported distension was noted and here the walls looked thinned, but where support was forthcoming these effects were absent. Careful examination of the alimentary tract from the cardiac orifice to the rectum revealed no abnormality, nor were any signs of recent or remote ulceration observed. The mucosa showed neither congestion, tendency to hæmorrhage nor excessive production of mucus. In like fashion the stomach was normal.

The only abnormality observed anywhere was patchy thinning of intestinal wall, a finding possibly to be correlated with the general emaciation of the patient and a condition of paralytic distension of coils of unsupported gut.

The weight of the spleen was 3½ ozs. The organ was small, firm in consistency and dark in colour. Microscopical section showed congestion increase in fibrous trabeculæ and large amounts of black granular pigment. (Such findings are indicative of malaria.)

The weight of the liver was 45½ ozs. The organ was almost of normal size, firm in consistency, pale in colour and slightly bile stained. Microscopical section showed fatty infiltration of liver lobules and a considerable quantity of golden and dark pigment deposit.

The gall bladder was distended with bile. The left kidney weighed 7, and the right 6 ozs. Both kidneys were tough, the cortex being somewhat narrowed and the capsules distinctly adherent. These macroscopical findings indicated a mild grade of chronic nephritis, while microscopical section showed an extensive desquamation of the tubular epithelium.

The pancreas was prominent, and firm in consistency but appeared normal on section. There were no pathological adhesions to adjacent viscera.

The root of the mesentery contained a number of firm, but not hard glands of oval or elongated appearance. Microscopical section of two of these glands showed no abnormality. The vessels at the edge of the mesentery—especially the veins—appeared prominent.

What was believed to be the *receptaculum chyli* was dissected out. It was found to be empty; its walls were soft and it looked normal. Vessels going into it looked soft and collapsed. Microscopical section showed a convoluted musculo-fibrous structure containing traces of a homogenous material (lymph chyle) in the lumen. No fibrosis was noted and the endothelial cells were normal. Gram-staining revealed no yeasts.

Another section of tissue from the region of the *receptaculum chyli* was also examined. It contained portion of a lymph gland and some large vessels—veins or lymph ducts—and also a portion of another vessel probably the wall of the *receptaculum chyli* itself. This consisted of loosely woven muscular fibres lined by flat endothelial cells. No pathological changes were noted in any of these structures and the lymph gland presented a normal histological appearance.

The sympathetic ganglia were also normal.

### C Bone-Marrow

Soft red marrow like blood clot (raspberry jam) filled the entire medullary cavity as well as all the trabeculæ of the cancellous bone of the tibia, the central canal of which had actually been enlarged by overgrowth and replacement by red marrow. The macroscopical appearances were identical with those seen in pernicious anæmia.

# THE MORBID ANATOMY OF SPRUE

BY

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SUMMARIZING our experience of the eight autopsies under review, we propose to describe in general terms the post-mortem appearances of an advanced case of sprue

The record of the individual autopsies are detailed in the Appendix at the end of the paper

Most of our cases were Eurasians and, owing to the great objection which this class of patient has to post-mortem mutilation, it was not found expedient to remove the brain and spinal cord or to carry out such a full examination of the skeleton as was required to show the complete distribution of aplastic or hyperplastic marrow in the long bones. As we have never met with clinical evidence of organic disease of the brain or spinal cord or of the peripheral nerves in any case of sprue during life, the omission of these organs from the necropsy would not appear to be a matter of much importance

On the other hand, a limited examination of the osseous system may occasionally lead to erroneous views regarding the real condition of the marrow, for as Archibald (1919) and Sheard (1924) have pointed out, erythroblastic response is occasionally entirely absent from one or more of the long bones though present in others, or in the flat or short bones

## *Weight of the Viscera*

We are not inclined to stress unduly the weight of the different viscera because there are factors which are difficult to assess in comparing them with standard weights in the textbook. The weight of a viscus is mainly of value when regarded in relation to body weight, and the Eurasians are generally more

The œsophagus appeared normal while the stomach presented some slight congestion of the mucous membrane.

The mucous membrane of the small intestine showed some patchy congestion and a healing ulcer was visible about 2 inches above the ileo-cæcal valve. This ulcer was superficial, of about  $\frac{1}{2}$  inch diameter with a greyish base, it presented an irregular circumference. The surrounding mucous membrane was slightly puckered though the edges were not indurated. Thinning of the intestinal wall such as is generally described in sprue was not noted.

The large intestine presented no abnormality beyond some patchy congestion of its mucous membrane.

The liver weighed 2 lbs (32 ounces). Adhesions were not present but there were irregularities and depressions of its antero-inferior edge. The gall bladder was almost empty containing only a small amount of brownish coloured bile.

The spleen weighed  $3\frac{1}{2}$  ounces, and presented marked signs of fibrosis.

The right kidney weighed  $4\frac{1}{2}$  ozs, and the left  $5\frac{1}{2}$  ozs. Both were apparently normal. There was no fibrosis and the capsules were not adherent. The supra-renals and bladder appeared normal, the latter being distended with urine.

The pancreas which weighed  $1\frac{1}{2}$  ozs, and the thyroid appeared normal. The parathyroid glands were not identified.

### *C The Osseous System*

The right femur was removed and sawn through longitudinally. Its structure and appearance were painted (Plate LIX). The cancellous tissue spaces in the neck of the femur and the medullary canal for about three inches were filled with red marrow. Below this level the central canal and trabeculae contained gelatinous lemon yellow material except for a small quantity of maroon coloured marrow localized in some of the lateral trabeculae in the middle of the femur. The trabeculae spaces and medullary canal in the lower third of the femur was entirely occupied by yellowish coloured marrow.

Microscopical examination of smears of the red marrow of the femur showed large numbers of myelocytes. Normoblasts and megaloblasts were also present, the latter being infrequent. In contra-distinction to the red marrow which was very cellular, the yellow marrow contained only fat cells and a few red blood corpuscles.

Cultures were also made for pathogenic yeasts. A yeast resembling *M. ashfordi* was isolated from the large intestine but negative results were obtained in cultures taken from the spleen, liver, pancreas, kidney, cancellous bone, small intestine and blood.

### SUMMARY

This patient showed a restricted area of terminal pneumonia. There was a small ulcer near the ileo-cæcal valve but no other noteworthy change was present in the intestine. The bone-marrow still showed hyperplasia of moderate grade similar to that observed in certain secondary anæmias, but the peculiar gelatinous appearance of the yellow marrow suggested that at an earlier period of the disease this hyperplasia may have been even more extensive than in its terminal stages.

**Case 5 N T Male.** Aged 27 years. Admitted 28th February, 1927, died 5th March, 1927, autopsy 12 hours after death.

**Clinical summary**—This case was not on our register but was well-known to the medical staff having been in hospital previously for sprue. He was re-admitted in a moribund condition and died shortly afterwards.

**Precis of post-mortem notes**—The patient was extremely emaciated without any sign of fat either subcutaneously or in the omentum. The bloodlessness was striking, almost none escaped during removal of the organs and even the heart contained but a little clot. The venous portal system contained a little dark grumous blood.

The tongue is nearly always wasted and its surface glazed and atrophic. The latter finding is often associated with disappearance of the lingual papillæ. Ulceration is only occasionally seen at autopsy, but earlier in the disease it is not an infrequent clinical phenomenon.

### *The Thorax*

Apart from a decrease in weight, the lungs usually present no abnormality. Both tuberculous and pneumonic lesions of a terminal nature were noted in our series.

The small weight of the heart has already been referred to in certain cases of sprue. Unlike pernicious anæmia, its muscle generally shows no signs of fatty or other degeneration. The size of the organ is small, the colour dark and the valves are normal. The vessels show no special tendency to atheroma.

### *The Abdomen*

When the abdomen is opened the intestines are generally observed to be emptied and collapsed, though there may be ballooning of some coils of the small bowel where they are unsupported by adjacent viscera. Often the intestines appear tenuous and semi-transparent and sometimes they are of a pinkish colour due to injection of the small vessels with blood. Signs of active inflammation are, however, absent. The liver and spleen are not visible until they have been drawn forward.

The naked eye changes in the alimentary canal are often varied and insignificant and fall far short of what would be expected from the clinical features of sprue. No findings designated as typical for every case can be described, but the pathological lesion most commonly observed in the gastro-intestine is an atrophic thinning of the gut wall. This occurred in 4 of the 8 cases under review.

The mucous lining of the œsophagus may be thin and pale with patchy injection of surface vessels, but more frequently it shows little that is abnormal.

The stomach likewise presents a normal appearance, though the presence of petechial hæmorrhages and focal congestion in the mucosa may be met with. In the duodenum ecchymoses, thickening of the mucous membrane, superficial erosions and degeneration of superficial epithelium may be met with in different cases and similar lesions are to be observed in the jejunum-ileum. Actual ulceration of the mucosa is uncommon. Once it occurred in the form of superficial erosions, and on another occasion an isolated superficial healing ulcer of about  $\frac{1}{2}$  inch diameter was met with 2 inches above the ileo-cæcal valve. Similar findings have been described by Fischer and Von Hecker (1922), Manson-Bahr (1924) and others, but owing to their inconstancy they can hardly be regarded as constituting the essential lesions of sprue. Congestion and atrophic changes in the surface epithelium may be observed in the colon, while its walls may be either thickened or thinned. In other cases the large intestine appears normal.

The liver is small, its average weight being 38.4 ozs. It shows no characteristic morbid changes and neither fibrosis nor fatty degeneration are obvious to



The structure of the duodenum was generally normal but some areas showed degenerative changes in the superficial epithelial cells and here the villi were 'withered' looking and lacking in epithelial covering. The jejunum presented similar features but the changes were more extensive, whereas the ileum showed them to a less degree. The appendix was normal.

The cæcum and large intestine show thickening of the mucous and submucous coats and some increase of the connective tissue elements, the muscular coats appear thinned and degenerate. There are no inflammatory changes, ulceration or erosion in the mucous membrane.

#### SUMMARY

There was a profound anæmia of aplastic type, associated with extreme emaciation and reduction in volume of all the viscera.

Slight changes in the intestinal canal, with some degeneration of its lining epithelium were also present.

Bilateral apical tuberculosis of the lungs occurred as a terminal complication.

**Case 6.** (S 80) D M Male Aged 32 years Admitted 16th February, 1927, died 16th June, 1927.

The patient gave a typical history of sprue and presented the characteristic signs as regards tongue and stools. Severe emaciation and anæmia were present. The blood condition was as follows —

R B C	= 1,762,500 per c mm
W B C	= 4,375 " "
Hb	= 40 per cent
C I	= 1 1
Polymorphs	= 55 2 per cent
Lymphocytes	= 42 8 " "
Large mononuclears	= 0 8 " "
Eosinophiles	= 0 6 " "
Transitional cells	= 0 6 " "

Films showed anisocytosis, a few microcytes and poikilocytes. No normoblasts were seen. The patient had recently lost two stone in weight, and left hospital against advice.

He was re-admitted on 10th June, 1927, extremely anæmic and almost moribund and died on the 16th June, 1927. During the three months since he left hospital he had rapidly gone downhill and appears to have had a blood crisis. His blood state was now —

R B C	= 400,000 per c mm
W B C	= 2,812 " "
Hb	= 10 per cent
C I	= 1 25 " "

The blood films were very thin and cells were scarce. The blood itself looked like water and left no visible film on the slide. In size the corpuscles were remarkably constant and only a few poikilocytes and megalocytes were seen. No nucleated reds were observed while polychromasia was scarce. Basket cells and cellular debris were abundant but platelets were scanty. The hæmatological findings indicated a profound anæmia without signs of regeneration of the marrow.

Blood transfusion was performed without avail.

#### PRECIS OF POST-MORTEM NOTES

The body was emaciated and presented no signs of decomposition. Rigor mortis was absent. The skin was waxy, white and glossy, the face slightly pigmented, a brownish-yellow hue. No pigmentation on the body was observed. The abdomen was slightly distended in its lower half. On section no subcutaneous fat was found. The muscles were

## MICRO-HISTOLOGY OF THE ORGANS IN SPRUE

*The Tongue*

The cases which come to autopsy are naturally advanced in the disease so that the tongue (*see* Plate LX, figs 1 to 6) generally appears smooth, glossy and atrophic with considerable destruction of the lingual papillæ of both filiform and fungiform types. There is not infrequently evidence of surface abrasion and occasionally of small ulcers which show little inflammatory reaction. In such cases there is often irritation down-growth of epithelium near the lesion. The mucous membrane is thinned and atrophied over most of the tongue especially at the tip and sides, but in some areas thickening and hypertrophy of the epithelial surface due to long continued mild irritation is seen. The whole organ appears wasted, but the muscular tissue shows no change.

In every case the sections have been specially stained for monilia and, in a few instances, some penetration of the mucosa with hyphæ of monilia have been seen. The majority of cases, however, have shown neither yeast invasion nor penetration by bacteria.

The principal changes in the tongue are thinning and atrophy of the mucous membrane and an absence of inflammatory reaction.

*The Œsophagus and Gastro-Intestine*

The Œsophagus was examined in several cases but no atrophy of the epithelial layers was found, monilia were not detected, nor any evidence of bacterial invasion during life.

The stomach was in most cases normal. In one or two instances there were patchy areas of congestion or of extravasation of blood from small capillaries, but the glandular structures were not shown to be atrophic or markedly degenerate.

The condition of the intestine (Plates LXI, LXII and LXIII) presents an interesting problem and one full of pitfalls. We have first to remember that incipient and sometimes well defined post-mortem changes are present in our specimens, and we have to guard against being misled by these appearances. All our autopsies were held in Bombay where no facilities for cold storage were available. Some were performed during the hottest part of the year when the physical conditions of the gut render it particularly prone to early decomposition. It is true that any case showing obvious signs of decomposition was excluded from this series but the early microscopic changes have still to be carefully considered from this viewpoint.

As a by-product of this enquiry we took the precaution of studying the post-mortem changes in the intestine of a test animal at various stages after death and a separate note on our results, together with photomicrographs of these conditions, are given in another paper\* for purposes of comparison. We are convinced from the careful study of a large number of sections that the conditions we describe are not those of decomposition but represent changes in the intestinal wall, the result or the accompaniment of sprue.

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\*The Microscopical changes occurring in organs after Death, pages 827—830

The jejunum looks normal under low power magnification but under oil immersion shows superficial necrosis of parts of the villi and a lot of fine granular pigment collected in these areas. This pigment does not give the hæmosiderin reaction.

The ileum shows superficial necrosis of villi and loss of staining reaction in cells deeper in mucosa. The submucous glands are in some areas markedly degenerated and broken up. The large intestine was normal.

#### SUMMARY

The pathological findings in the small intestine suggest the presence of a locally absorbed toxin producing damage or destruction to the lining epithelium of such intensity as to markedly interfere with its secretory and absorptive functions. In like manner the areas of degeneration in the supra-renal glands indicate the selective action of some circulatory toxin on the cortical cells. The bone-marrow was in a hyperplastic state resembling that of pernicious anæmia. Except for this latter feature the morbid appearances were those of advanced sprue.

**Case 7** (S 87) J F European Aged 40 years Female Admitted 9th May, 1927, died 16th July, 1927.

There is a history of a gradual development of sprue symptoms during the last two years. The weight which was 13 stone some years ago actually sank to 5 stone 12 lbs, shortly before death.

The patient presented the classical clinical syndrome as regards her tongue, digestive tract and stools. She was emaciated and extremely anæmic. In the wards she was considered to have terminal phthisis but at the autopsy this clinical diagnosis was not confirmed. She died on 16th July, 1927.

The blood findings on the 20th May, 1927, were as follows —

R B C	= 1,937,500 per c.mm
W B C	= 5,000 " "
Hb	= 50 per cent
C I	= 13
Polymorphs	= 74.4 per cent
Lymphocytes	= 21.8 " "
Large mononuclears	= 1.6 " "
Eosinophiles	= 0.4 " "
Transitionals	= 1.4 " "
Mast cells	= 0.4 " "

The blood film showed a megalocytic anæmia with a few microcytes and poikilocytes. No nucleated reds and very little polychromasia were observed, i.e. signs of regeneration were minimal.

#### PRECIS OF POST-MORTEM NOTES

Emaciation, pallor of skin, and bloodlessness of all internal organs were present. Some general diffuse pigmentation of face but not of skin elsewhere was observed. The blood was thin and watery. The liver was large (55½ ozs), and was soft and fatty in consistency, whereas the spleen was somewhat reduced in size (4½ ozs).

The left lung was collapsed and bound down by old adhesions. It was small and fibrous weighing only 7 ozs.

The right lung was large and voluminous (32½ ozs) and filled nearly the whole chest. compensatory emphysema was present.

The kidneys weighed 3 ozs each, were somewhat contracted and showed some interstitial change.

The stomach was normal.

The duodenum showed mucous membrane which was thickened and like velvet pile, but no erosion or ulceration was observed.

Some further details concerning the micro-histology of individual cases will be found in the case records appended to this paper (pages 812—825) and a study of the photomicrographs (Plates LX to LXIV)

The bacteriological features of the intestine were interesting as they showed, especially in the ileum and the colon, what appeared to be an invasion of bacteria deep into the substance of the mucosa (Plate LXIV, figs 5 and 6)

The bacteria seen in such situation were not the large Gram-positive bacteria of putrefaction, but principally cocci and some thin Gram-positive bacteria as well as Gram-negative coliforms. When post-mortem changes were evident the large putrefactive bacteria were also present, but were scattered throughout the gut wall and not predominantly in the mucosa. The cocci and other bacteria referred to were almost entirely restricted to the mucous membrane, few having penetrated to the submucosa and none to the muscular or serous coats. From these features we are led to conclude that there is an ante-mortem invasion of the gut wall with bacteria. Recent researches on bacterial invasion of the intestinal wall as a result of vitamin C deficiency strengthen us in our opinion that a similar process occurs also in sprue.

Such findings suggest that there may be two distinct ætiological factors in the production of the sprue syndrome, firstly, a specific virus\* acting on the intestinal tract and resulting in degeneration, atrophy and diminished resistance of the mucosa to microbic invasion, and, secondly, *secondary* bacterial invaders and their soluble toxins which give rise to a chronic toxæmia resulting in a further atrophy of the parenchyme cells in the viscera and of the erythroblastic tissue in the bone-marrow. As has been detailed in another paper on the 'Bacteriology of Sprue,' published in this Journal (*see* Vol XVI, No 1, July 1928, pp 95—108), we have not been able to isolate any specific bacterium, but in the later stages of the disease the above hypothesis suggests that the primary virus may be less in evidence. Actually at this period hæmolytic bacteria both aerobic and anaerobic are commonly found not only in sprue fæces but also in the contents of the upper part of the intestinal tube. When associated with atrophy of the mucosa and secondary bacterial invasion, changes in the intestinal flora of this nature may have considerable significance, and it is not difficult to concede that they may be related ætiologically to the hypoplasia and aplasia of the bone-marrow so characteristic of the later stages of this disease.

#### *The Liver*

The liver (Plate LXIV, figs 1, 2 and 4) in some cases was normal but in others there were definite signs of fatty change, cloudy swelling and disintegration of the parenchyma in diffused area. There was no overgrowth of interstitial tissue and no change in Kupffer's cells or in Glisson's capsules. No monilia or bacteria were ever detected in sections of the liver.

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\* Ashford and the Porto Rico school maintain that *Monilia psilosis* is the primary ætiological factor in sprue. We disagree with this view though we concede that a changed intestinal flora, associated with increase in yeasts, may possibly contribute to the production of such clinical features as abdominal distension, intestinal flatulence and frothy stools.

before further examination could be carried out The blood condition on the 17th June, 1924, was as follows —

R B C	= 3,412,000 per c mm
W B C	= 7,500 " "
Hb	= 75 per cent
C I	= 1 1
Polymorphs	= 58 8 per cent
Lymphocytes	= 37 6 " "
Large mononuclears	= 1 0 " "
Eosinophiles	= 0 6 " "
Transitionals	= 2 0 " "

The blood picture showed anisocytosis but little change otherwise No normoblasts were seen

#### PRECIS OF POST-MORTEM NOTES

The body was emaciated but fat was still present in the omentum and around the abdominal viscera Rigor mortis was present The skin was pale and some pigmentation of the lower part of the abdomen, but not of the face, was observed The liver and spleen were not visible on opening the abdomen The intestines appeared normal

The liver weighed 46 ozs, and appeared normal

The spleen was 4½ ozs, and presented no abnormality

The pancreas was normal

The heart weighed 12 ozs, and was covered with fat

The lungs and larynx were normal

*Bone-marrow*—Fatty marrow was present throughout the tibia, only a few red points being seen here and there The condition is one of aplastic anæmia Very little blood was seen in the viscera or vessels during the autopsy

The tongue was pale, flabby and atrophic

The stomach showed extensive ecchymoses

The duodenum presented thickening of its walls especially of mucous membrane layer which also showed some superficial erosions Its surface was pitted and velvety but no actual ulcers were seen

The small intestine was congested and showed areas of petechial staining but no other obvious change in the mucosa was observed

In the large intestine the walls were slightly thickened but the mucous membrane itself was normal

The kidneys showed some interstitial change

#### HISTOLOGICAL CHANGES

The liver was fatty and pigmented Fatty degeneration of some of the cells with cloudy swelling, and a quantity of fine granular pigment generally distributed were observed Some pigment gave the prussian blue reaction but it was also equally evident in ordinary tissue sections

The spleen showed a thickened capsule while the parenchyma was congested and contained a quantity of melanin-like pigment

Of the kidneys one showed an adenomatous graft near the cortex, as well as some increase in interstitial tissue

The lungs contained sero-fibrinous exudate in the alveoli unassociated with cellular elements and corresponding with the œdema and congestion seen at the autopsy

The pancreas was normal

In the heart some cloudy swelling of the muscle fibres was observed while fine granular pigment was noted in many of the cells

significance At the same time in attempting to correlate such changes with the symptoms of adrenal deficiency, we have to remember the general principle that extensive anatomical changes in an organ are not necessarily associated with a demonstrable lack of physiological activity, and conversely that profound depression of function may find no anatomical expression even after the most careful histological examination

The microscopical changes observed in the *heart* were never so constant or well defined as those of pernicious anæmia The most common feature was the presence of fine pigment deposit in the muscle fibres resembling that seen in brown atrophy Fine granules of hæmosiderin and a melanin-like pigment were also observed in the most atrophic heart of the series (weight =  $2\frac{1}{2}$  ozs) and here the muscle fibres also showed cloudy swelling, vacuolation and fatty changes As a general rule, however, such stigmata of degeneration were absent

The pancreas which was sectioned in five instances showed no evidence of chronic interstitial pancreatitis or other morbid changes, and on pathological as well as biochemical grounds we find ourselves unable to support the idea that this organ is specially implicated in this disease

In view of Scott's theory (1923) of parathyroid deficiency in sprue, the parathyroid glands were dissected out in three cases Microscopical sections presented no abnormality

The abdominal *lymphatic* system including the *receptaculum chyli* was examined minutely in one case in view of the statement made by Davenport Jones (1924) that the lacteals were seen to be distended in a sprue patient operated on by him for appendicitis His suggestion was that there was some blocking of the efferent lymphatic system with consequent interference with the absorption of nutrition from the intestine The mesenteric lymph glands at autopsy, and also the receptaculum appeared normal, nor were signs found in the large lymph vessels to suggest any interference with the lymph flow

The hæmolymp glands were hyperplastic but otherwise their histology appeared normal Histogenous macrophages containing engulfed red blood corpuscles were not noted, whereas in pernicious anæmia they are a common finding

The microscopical changes in the *bone-marrow* in films, as well as in decalcified sections, bore out the naked eye appearances of aplasia and hyperplasia figured in Coloured Plates LVIII and LIX In the advanced hyperplasia seen in Cases Nos 3 and 6, great activity and cellular proliferation of the marrow elements were observed Myelocytes as well as large numbers of megaloblasts and nucleated red cells were demonstrated Pigmentary deposits were also in evidence The condition was indistinguishable from the megaloblastic degeneration of the bone-marrow originally described by Ehrlich as pathognomonic of pernicious anæmia As a rule, however, a condition of hypoplasia or aplasia existed The gelatinous yellowish material which filled the spaces in the cancellous tissue and the medullary canals of the tibia and femur consisted almost entirely of fat and left no cellular deposit after treatment with fat solvents such as ether A few nests of mature red cells were observed in one or two instances, but the associated nucleated reds

## EXPLANATION OF PLATE LVIII

### *The Bone-Marrow in Sprue*

- Case No 1* —The spongy tissue at the ends of the femur as well as its central canal are filled with greenish yellow marrow. One small focus of red marrow is located at its lower end. Definite aplasia is present.
- Case No 3* —There is intense hyperplasia of the bone-marrow similar to that described originally by Cohnheim in pernicious anæmia. Throughout the tibia all the spaces in cancellous bone as well as the enlarged medullary canal are tightly packed with marrow of the consistency and colour of raspberry jelly. Microscopical examination showed intense cellular proliferation of the marrow elements with the production of numerous megaloblasts as well as nucleated red cells.
- Case No 8* —(S 88) Except for a few points of red marrow the pale yellow variety is distributed throughout the tibia both in the cancellous tissue spaces and the central canal. Microscopical examination shows only fat cells. The bone-marrow is in a condition of advanced aplasia.
- Case No 9* —A case of sprue which was complicated before death by extensive tuberculosis. The bone-marrow of the tibia was completely aplastic.

aplastic anæmia, glandular deficiencies and other metabolic disturbances described as complicating advanced sprue

In respect of McCarrison's (1921) researches on the effect of vitamin deficiency as a factor in the production of bowel disease, one of us (F P M) has recently published a paper\* on this subject. The condition set up by a complete absence of vitamin C in monkeys produced a state of affairs which cannot be identified with sprue, but that is not to say that subminimal amount of vitamins over a length of time cannot at least contribute to the conditions which we have described in this paper. On this subject we keep an open mind being well aware from personal observations in Bombay that the incidence of sprue and its distribution in the community are difficult to reconcile with any hypothesis of dietary deficiency unless it be based on decreased absorption rather than deficient intake of vitamins in the diet.

#### SUMMARY AND CONCLUSIONS

1 The changes in the tongue are those which have already been described by other observers. The mucous membrane is thinned and atrophic and the lingual papillæ may disappear. There is desquamation of the epithelial layers and occasionally loss of tissue amounting to superficial ulceration with little sign of inflammatory reaction.

2 The changes in the intestine are most marked in the ileum, but are present to some degree throughout the whole tract. They are chiefly those of thinning and atrophy of the mucous membrane with marked degeneration and ultimately the almost complete disappearance of the absorptive and secretory epithelium. Here, again, the change is one of degeneration and aplasia, and if it is preceded by inflammation there is little evidence of such in the terminal stages of the disease. There is evidence of blood destruction in the mucosa suggesting the absorption of some hæmolytic substance from the intestine and the destruction of blood *in situ*.

3 The disposition of micro-organisms in the gut wall favours the view that there is *ante-mortem* invasion by bacteria such as is known to occur in conditions of malnutrition due to vitamin deficiency.

4 A specific atrophy of cardiac muscle out of all proportion to any decrease explicable in terms of mere starvation was noted in two cases of our series. On the other hand, the depreciation in the weights of the other viscera—notably that of the liver, spleen and kidneys—can be explained on the latter basis.

5 The liver, kidney and the adrenals sometimes show microscopical changes of a degenerative nature such as might be induced by the action of a toxin. Iron pigment is laid down in moderate amount especially in the liver, but not in the same quantities as characterise pernicious anæmia.

6 The bone-marrow as seen in the femur or tibia shows in most cases marked aplasia. The red marrow where present is much reduced in quantity,

\* 'The Association of Bowel Disease with Vitamin C Deficiency,' *Ind Jour Med Res*, Vol XVI, No 1, July 1928, pp 77-94  
J, MR



## EXPLANATION OF PLATE LIX

### *The Bone-Marrow in Sprue*

- Case No 4*—An example of moderate grade hyperplasia of the bone-marrow occurring in sprue. The cancellous tissue spaces in the neck of the femur and the upper part of the medullary canal are filled with red marrow, while below this level the yellow variety is mostly evident. Microscopical examination of the latter shows fat cells and a few red blood corpuscles, whereas myelocytes, normoblasts and scanty megaloblasts characterize the former.
- Case No 5*—The lower end and shaft of the femur shows a uniform distribution of gelatinous yellow 'apple jelly' marrow. In one small area near the head a focus of red jellified marrow is noted. Microscopical examination showed the yellow marrow to consist almost exclusively of fat cells, only a few nests of mature red cells and scanty normoblasts being observed. As in Case 7 the aplastic condition of the marrow is evident.
- Case No 6*—(S 80) Soft red marrow fills the cancellous tissue spaces and the medullary canal of the tibia throughout its entire length. The hyperplasia is of similar grade to that observed in Case 3, resembling the bone-marrow of pernicious anæmia.
- Case No 7*—(S 87) This specimen shows advanced aplasia. Only one small patch of red marrow was present throughout the whole length of the tibia and this was jellified. The shaft of the bone was decidedly thinned and the pale yellow marrow was found to consist exclusively of fat cells and large acellular spaces when examined microscopically. Normoblasts and myelocytes were present in smears of the red marrow, but megaloblasts were not numerous.

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## EXPLANATION OF PLATE LX

### *The Tongue in Sprue*

(Magnifications approximately  $\times 100$ )

- Fig 1 Shows irritation and slight keratinization of surface epithelium
- 2 Shows irritation downgrowth of epithelium from an active lesion on the tongue
- 3 Shows a condition of leucoplakia and atrophy of the epithelial structure
- 4 Shows irritation and keratinization on the left and a thinning of the epithelial layer on the extreme right of the section
- 5 Shows thinning and atrophy of the epithelial layer with surface abrasion and without inflammatory reaction
- 6 A photograph from a composite drawing showing monilia downgrowth into the epithelial layer in different situations (The original drawing was done with an oil immersion lens)

The right kidney weighed 3.5 and the left 3 ozs. The former appeared normal, while the latter was lobulated and showed some congestion of the striated vessels. No evidence of chronic nephritis was observed.

The uterus was large and flabby and on incision contained a mass of dark clot adherent to its walls, as well as some dark grumous blood. No evidence of post-mortem infection was observed.

#### *C Bone-Marrow*

The shaft of the femur was examined. Its central canal as well as all the spaces in the cancellous tissue of the head and lower end of the femur were filled with greenish-yellow marrow. Only one small focus of red marrow was found situated about three inches from the lower end of the bone. This finding is typical of the severe aplastic anæmia met with in many fatal cases of sprue.

#### *D Tongue*

This organ showed well marked atrophy of the mucosa at the tip and sides, but the papillæ on the dorsum and back of the tongue were well in evidence.

Sections of the tip of the tongue stained by Gram's stain presented a coarse mycelium penetrating vertically downwards through the stratified layer of epithelium. The sections were very thick and the mycelium was unstained. Similar appearances were noted with hæmatoxylin and eosine, and Van Gieson, none staining the mycelial elements.

Fragments of mycelia were also noted in the middle of the tongue, but not at its base.

Ziehl-Nielsen showed that the mycelium was not acid-fast, but took the counter-stain. The yeasts seen in partially detached epithelial scales were regarded as suitable for photography.

#### SUMMARY

The case was definitely one of acute sprue with atrophy of the tongue, low grade intestinal changes, and a severe degree of aplastic anæmia.

**Case 2** G. K. Anglo-Indian. Age 33 years. Male.

This patient was in St. George's Hospital with malaria and dysentery in September and October, 1923, also in January 1924. He was treated with quinine and intramuscular injections of emetine hydrochloride.

On 14th February, 1924, he was re-admitted with history of apyrexial diarrhœa, sore tongue, and abdominal pain which was severe. The edge of the tongue was red and sore. The bowels were open 2 to 6 times daily.

The blood picture was as follows —

R.B. Cs	=	4,800,000 per c.mm.
Hæmoglobin	=	85 per cent
C I	=	0.89

He was treated as sprue, emetine being administered, and was discharged on 26th February, 1924.

He was re-admitted on 18th May, 1924, with apyrexial diarrhœa, sore tongue and extreme emaciation. The stools numbered 5 to 8 daily and were typical of sprue.

Throughout the patient proved difficult to treat. His weight was only 5 stone 7 lbs., and he died on 27th May, 1924, when autopsy was performed.

#### AUTOPSY

An emaciated man with extensive tattoo markings on the skin.

#### *A Thorax*

The heart showed no abnormality, while the lungs presented a few old pleural adhesions.

## EXPLANATION OF PLATE LXI

### *The Small Intestine in Sprue*

(Magnifications approximately  $\times 100$  )

- Fig 1 Duodenum shows extensive withering of villi and general atrophy of absorptive and secretory structure
- „ 2 Jejunum shows similar changes especially in the villi on the right of the section
- „ 3 Jejunum shows more advanced changes of the same nature and general thinning of the gut wall
- „ 4 Ileum shows similar changes
- „ 5 Ileum shows small celled infiltration of one group of villi and withering of another group
- „ 6 Ileum shows a part where the submucous glands are abundant but these show degenerative changes also

The differential count was as follows —

Polymorphs	= 65 per cent
Lymphocytes	= 21 " "
Large mononuclears	= 7 " "
Transitional leucocytes	= 5 " "
Eosinophiles	= 2 " "

The blood films showed some megalocytes but no nucleated reds, and poikilocytosis and polychromatophilia were noted

The patient ran a temperature for 36 hours, and the bowels were open 2 to 4 times daily throughout the 18 days in hospital. During this time he appears to have gained a stone in weight under treatment with parathyroid, calcium lactate and sprue diet.

The weight was recorded as being 6 stone 9 lbs on the 15th April, 1924, and 7 stone 9 lbs on the 29th April, 1924. He was discharged on this date when the stools were of better colour and semi-solid in consistency. The tongue also had vastly improved.

He was re-admitted on 3rd August, 1924, being brought in by police in a collapsed condition—very ill. He was then pulseless with a subnormal temperature and diarrhoea, and died ten days later, i.e., on the 13th August, 1924. The stools were described as large, pale, frothy and foul smelling, from 4 to 6 daily and chiefly occurring during the early morning hours. The tongue showed a few red patches of inflammation.

Blood count on 8th August, 1924, showed much increase in the anæmia since last admission.

Hæmoglobin = 45 per cent (previously 70 per cent)

Red Blood Corpuscles = 2,400,000 (previously 4,000,000)

Colour Index = 0.94 ( " 0.9 )

Leucocytes = 5,625 per cmm ( " 6,250 per cmm )

The differential count was as follows —

Polymorphs	= 68 per cent
Leucocytes	= 18 " "
Large mononuclears	= 9 " "
Transitionals	= 4 " "
Eosinophiles	= 1 " "

The blood smears showed poikilocytosis and polychromatophilia. Basophilic degeneration was noted, but only two nucleated red cells (erythroblasts) were seen.

#### SUMMARY

This case was not on our register but on clinical grounds there can be no doubt that he was suffering from genuine sprue. He was usually admitted in a serious condition but immediately he showed the slightest signs of improvement he used to leave hospital. Each attack was more severe than the previous one, and being well advanced in years such a termination was inevitable. The case is of special interest owing to the bone-marrow findings.

#### AUTOPSY

13th August, 1924, the body was that of an elderly man in an extremely emaciated condition. Death had occurred some 6 to 8 hours previously. There was no yellow colour or bile staining of the tissues.

#### A Thorax

The right lung weighed 25½, and the left 19½ ozs. The bases of both lungs were very congested and markedly œdematous—almost of semi-solid consistency. The apices and edges were emphysematous.

## EXPLANATION OF PLATE LXII

### *The Small Intestine in Sprue (continued)*

- Fig 1 Ileum showing well marked small celled infiltration of the mucous membrane representing an earlier stage of the degeneration process
- „ 2 Colon showing extensive withering of the epithelial structures lining the gut
- „ 3 Villi of the jejunum showing early degenerative changes including destruction of epithelial cells and diffuse degeneration of the body of the villus
- „ 4 Villous surface of the ileum showing small celled infiltration and degenerative changes in the epithelium
- „ 5 Similar changes in the ileum of another case showing small celled infiltration of the mucosa
- „ 6 A degenerate or withered villus in the ileum  
(Figs 1 and 2 under magnification at approximate  $\times 100$ )  
(Figs 3 to 6 under magnification at approximate  $\times 450$ )

Smears of the bone-marrow stained with Romanowski showed great activity and cellular proliferation of the marrow elements with large numbers of megaloblasts and nucleated red cells. Small collections of greenish-brown pigment were also observed. The so-called 'Megaloblastic degeneration of Ehrlich' was a characteristic feature of the preparation.

#### SUMMARY

The case was one of special interest as it definitely illustrates a case of sprue coming to autopsy at a state when the condition of the bone-marrow was indistinguishable from that of pernicious anaemia. Apart from the clinical history of the case the extreme emaciation, the absence of yellowish discolouration of somatic muscle, and a heart (11½ ozs) unassociated with myocardial degeneration constituted features which enabled these two diverse conditions to be differentiated at autopsy.

**Case 4.** W. M. Anglo-Indian. Aged 24 years. Male.

This case came to autopsy on the 9th May, 1925, and a coloured drawing was made of the bone-marrow. No detailed blood counts were done.

On the 13th January, 1925, he was admitted suffering from diarrhoea which had persisted for two weeks. The bowels were open 4 times daily and the stools were described as being whitish in colour, loose and foul-smelling. There was no fever, but the tongue was sore. Dyspnoea was present as well as some ascites and oedema of the legs, the latter had been present for 8 days. The urine showed some albumen.

The patient was treated with digitalis and was discharged on the 31st January, 1925.

He was re-admitted on the 18th February, with oedema of the feet, diarrhoea and pain in the right iliac fossa, the temperature being practically normal and blood slides negative for malaria parasites. The weight was 5 stone 11 lbs. The patient was discharged on the 15th March, 1925.

He was re-admitted on 3rd April, 1925, complaining of headache, vomiting and fever (?). There was a slight rise of temperature on one day but blood films were negative. He was discharged at his own request on the 20th April, 1925.

He was re-admitted on the 8th May, with severe diarrhoea. The patient was very debilitated and died forthwith.

#### SUMMARY

This case did not appear in our register, but on clinical grounds the case was definitely regarded as one of chronic sprue.

#### AUTOPSY

Patient died 11 p.m. on the 8th May, 1925, and autopsy was performed about 12 hours later, i.e. 11 a.m. on the 9th May, 1925.

The body was emaciated and pale. No oedema was present. There was a nævoid-like condition on the forehead.

#### A. Thorax

**Heart**—The organ weighed 8 ounces. The muscle was somewhat pale and flabby but the valves were not diseased. The cavities contained no blood clot and the aorta showed no signs of atheroma.

**Lungs**—The left lung weighed 1 lb (16 ozs) and the right 1½ lb (24 ozs).

The lower portion (1½) of the base of the right lung was consolidated, the visceral and parietal pleura being adherent to the diaphragm in this region. On section the surface appeared consolidated and granular while smears showed pneumococci but no tubercle bacilli. Small pieces of this tissue sank in water. The remaining portions of the left lung as well as the right lung appeared normal.

#### B. Abdomen

The tongue was somewhat atrophic in appearance though the papillae were fairly well marked. No rawness nor excoriations were demonstrated.



### EXPLANATION OF PLATE LXIII

#### *The Large and Small Intestine in Spine (continued)*

(Magnifications approximately  $\times 450$  )

- Fig 1 More advanced change of the ileum showing a group of submucous glands which are apparently normal
- „ 2 Similar changes in the ileum where the glands show early degenerative changes
- „ 3 Also the ileum with advanced withering of villi and degeneration of the glands of Lieberkühn
- „ 4 A vessel in the submucosa containing many black pigment granules which do not give the prussian blue reaction for hæmosiderin
- „ 5 The mucosa of the colon showing advanced withering of the villi
- „ 6 Also the lower layers of the mucosa of the colon showing breaking up of the glandular element

On opening the abdomen the intestines were seen to be empty, their coats looked thin and the whole appeared pink as if injected but this may have been due to the thinness of the walls. The liver and spleen were not visible. On incising the intestines the small bowel showed some petechial points but no ulcers or demonstrable surface erosions. The mesenteric glands appeared to be enlarged but that may have been due to absence of fat. The large intestine, especially the sigmoid, was definitely thickened and suggested chronic inflammation. There were no ulcers but the mucous membrane was thickened and soft and showed surface erosions. There was a history of recent colitis before death as well as the presence of typical sprue stools.

The liver (27½ ozs), was reduced in size and of darkish colour but otherwise normal. The spleen was small, (3½ ozs), dark and wrinkled.

The right and left lungs weighed 14½ and 12 ounces respectively. They showed recent, limited but active cavitation in both apices and tubercle bacilli were present. This was probably a terminal infection superadded to a profound condition of sprue with aplastic anæmia.

The heart weighed only 4 ozs. It was pale but showed no fatty change or other sign of degeneration.

The femur was removed and cut lengthways. The lower end and shaft were filled with a gelatinous yellow substance like apple jelly and in one small patch near the head there was a red jelly-like area. The condition of the marrow on the whole was that of a well marked aplastic anæmia.

The organs unless otherwise referred to were normal. The right and left kidneys weighed 3 ozs each.

#### HISTOLOGICAL APPEARANCES

*Liver*—There is a considerable amount of golden yellow pigment within the liver cells especially around the hepatic vein and the centre of the lobules. The pigment is readily seen in ordinary sections and is not rendered more evident by staining for iron. The liver cells are somewhat cloudy and diffuse but nuclei are normal. The capsule is definitely thickened. Kupffer's cells are normal while Gram's stain reveals no bacteria or yeasts.

*Spleen*—Thickening of the capsule and increase of fibrous trabeculae throughout the organ was found. There was also congestion and aggregation of the red blood corpuscles which were in various stages of dissolution. Large amount of dark and golden-yellow pigment were seen throughout the organ more particularly in certain areas.

The pancreas was normal and the islands of Langerhans well marked.

The apex of the lung shows recent active tuberculosis.

The kidneys are intensely congested and show desquamation of the tubular epithelium but are otherwise normal.

The mediastinal lymph glands show recent tuberculous infection.

The heart muscle shows much fine golden pigment in all its fibres, but the muscle substance is not fatty or degenerate.

*Bone-marrow*—In fresh films and in stained sections, most parts were aplastic but some foci of active development were observed. The red blood corpuscles were nearly all mature and collected into groups very few being scattered throughout the marrow. Nucleated reds were scarce. The ordinary corpuscles showed no polychromasia or stippling. Myelocytes and granular cells were scanty. The wide areas of gelatinous bone-marrow consisted of fat cells without any trace of blood-forming tissues.

The hæmolymph glands show hyperplasia with much mitotic and nuclear activity. The red blood corpuscles are numerous but most are deformed or fragmented and signs of regeneration are scarce.

*Alimentary canal*—The tongue showed a superficial abrasion or small ulcer without signs of inflammatory reaction around it. Marked irritation downgrowth of the epithelial layer was noted in some places but there was no evidence of monilia or bacterial invasion.

EXPLANATION OF PLATE LXIV  
*Certain Visceral Lesions in Spine*

- Fig 1 Degeneration of liver cells with overgrowth of connective tissue  
„ 2 Fatty and degenerative changes in the liver cells  
„ 3 Ditto in the adrenal cortex  
„ 4 Deposit of iron in the liver  
(All the above are under magnification approximately  $\times 450$ )  
„ 5 Bacteria which have invaded the deeper layers of the mucosa of the ileum  
„ 6 Ditto in the deeper layers of the colonic mucosa  
(Nos 5 and 6 under magnification approximately  $\times 1000$ )

pale The cæcum and ascending colon were dilated, while the intestines were thin and bloodless but no signs of inflammation were observed A little free fluid was present in the peritoneal cavity and the pericardial sac contained a large amount of straw coloured transudate The mesenteric glands were prominent and congested

*Thorax*—The heart weighed only  $2\frac{1}{2}$  ozs, and contained a very small quantity of blood There was œdematous connective tissue hanging from the apex and extending along the fissures

Fluid was present in both pleural cavities, while the lungs were emphysematous above and congested and œdematous in lower lobes

*The viscera*—The liver weighed 36 ozs, and was small and pale

The spleen weighed  $6\frac{1}{2}$  ozs, and was firm, dark and pigmented

The pancreas weighed 2 ozs, and was well defined, in consistency it was firm

The kidneys weighed  $4\frac{1}{2}$  ozs each Both showed slight reduction of the cortex and adhesion of the capsule The supra-renal cortex presented small opaque-whitish areas

*Alimentary canal*—The tongue was pale, smooth and glossy A few ecchymoses were noted in mucous membrane of the stomach Extensive ecchymoses in or below mucous membrane were present but no signs of inflammation or erosion were observed in the duodenum and jejunum

The ileum shows some areas of velvety mucous membrane which were thickened and a little congested The coats of the large intestine were thin, but the mucous membrane itself was normal The prussian blue reaction was well marked in the liver, slight in spleen and kidneys

*Bone-marrow*—The red marrow of the ribs showed little abnormality Throughout the length of the tibia, however, it was red, soft and markedly hyperplastic resembling that originally described by Cohnheim as characteristic of pernicious anæmia

#### HISTOLOGICAL APPEARANCES

No change in the structure of the liver was noted but an excess of hæmosiderin was present through the organ The sinuses of the spleen were congested and full of red blood corpuscles The serous covering was thickened and the trabeculæ present in excess There was abundance of dark or golden pigment scattered throughout the organ but only a part of it gave the prussian blue reaction The rest appeared to be allied to the melanins or some similar pigment.

The pancreas showed nothing abnormal The muscle fibres of the heart are cloudy and vacuolated and show some fatty degeneration They contain fine granules of hæmosiderin, some of a melanin-like pigment and some suggesting the appearance of brown atrophy

The lungs were normal

The bone-marrow shows a number of nucleated red cells but mature red blood corpuscles are not numerous Granular cells, myelocytes and polymorphs were numerous Some cells contain dark melanin-like pigment.

The hæmolymp glands are active and contain many young red blood corpuscles in the sinuses Some hæmosiderin and melanin-like pigment were present

The kidneys contain iron pigment but no change otherwise Small areas in the cortex of the supra-renals are present which are either degenerated or show an absence of normal cells Such findings suggest the action of some toxin No excess of pigment was noticed

*Alimentary canal*—The tongue examined at its tip, sides and root shows in some places thinning and degeneration of epithelial covering and small surface erosions and in others irritative downgrowth of epithelium and cornification of superficial layer No penetration by bacteria or monilia was detected

The duodenum shows desquamation and disappearance of surface epithelium in some areas even extending down to submucosa here and there The submucous glands stain diffusely and appear to be disintegrating



In the small intestine the coats were very thin and transparent while the mucous membrane was smooth and atrophic.

In the large intestine the coats were also thinned and the blood vessels injected but no obvious change was noted in lining membrane

The heart was flabby and pale but without any signs of 'tabby cat' striation

The bone-marrow of the tibia presented advanced aplasia. Only one small patch of red marrow was present throughout its whole length and this was soft and jelly-like. The shaft of the bone was decidedly thinned.

#### HISTOLOGICAL CHANGES

In the liver the cell outlines were blurred and presented some fatty changes. Areas of partial cell degeneration were present but there was no increase of interstitial cells. Some fine granular pigment was noted but it was much less than that seen in pernicious anaemia.

The left lung was collapsed and fibrosed and signs of tuberculosis were absent.

The heart muscle presented no sign of degeneration but pigmentary changes as in brown atrophy were present.

The pancreas was normal.

The spleen was engorged and its sinuses contained mutilated red blood corpuscles undergoing destruction. No hæmosiderin but a quantity of melanin-like pigment was found.

The kidneys showed some increase of interstitial tissue. The tubular epithelium was desquamating and the nuclei showed defective staining as in toxic nephritis. Some glomeruli were sclerosed, iron pigment was seen in the cells.

The supra-renals contained groups of cells in the cortex which were vacuolated and partly disintegrated. The histological appearances suggest a toxic origin for the condition.

In the yellow marrow no marrow cells or red blood corpuscles were seen, the microscopical picture consisting entirely of fat cells and large acellular spaces. Smears of the red marrow, however, still indicated foci of activity. Normoblasts were fairly numerous many of the red cells being of normal size and shape but megalocytes were not common in the specimens studied.

*Alimentary canal*—The duodenum at the pyloric junction and two lower levels shows degenerative changes in the superficial layers of mucosa and withering of villi. No bacterial or monilial invasion of living tissues was demonstrated.

The jejunum shows marked withering of villi and degeneration and shedding of superficial epithelium so that only the bases of the follicles are left intact. No definite ulceration or inflammatory changes have occurred.

The large intestine shows microscopical changes similar to those in the jejunum. Numbers of large slightly curved Gram-negative bacilli are to be seen penetrating the follicle and superficial layers of mucosa. Inflammatory reaction is conspicuous by its absence.

#### SUMMARY

This is a well marked case of advanced sprue presenting aplasia of the bone-marrow and thinning of the coats of the intestine. The mucous membrane of the small gut shows degenerative and toxic changes.

**Case 8** (S 88) R. W. Anglo-Indian. Aged 59. Admitted 13th June, 1927, died 4th August, 1927. Male.

#### CLINICAL SUMMARY

This patient was regarded as a typical case of chronic sprue which had slowly developed during the last three or four years. He was anæmic and wasted and had lost four stone in weight during the last year. He improved under treatment and was discharged from hospital but was re-admitted on 4th August, 1927, in a moribund condition and died.

*Series II* (three hours after death)

*Small intestine* (both levels) —No change visible under the low power. Under high power, the cytoplasm of the epithelium is granular and stains somewhat diffusely. The edges are blurred as in cloudy swelling, but the nuclei are prominent and a little swollen but stain well. The muscular and submucous coats are unaltered. The villi are not 'withered,' nor is there any sign of auto-digestion and the epithelial cells are intact. Bacteria are abundant in the faecal matter in the lumen and a few are seen within the villi near to their free margin. No invasion of the mucosa with bacteria and no large Gram-positive putrefactive organism seen.

*Large intestine* shows similar changes but in a less degree. No invasion of the mucosa by bacteria.

*Kidney* —The tubular epithelium is swollen and cloudy and cytoplasm broken up into fine granules. The nuclei are swollen but stain well. No bacteria seen.

*Liver* —Similar changes in the cytoplasm of the liver cells. No bacteria seen.

*Series III* (6 hours after death)

*Small intestine* (both levels) —Similar changes in the cytoplasm to those already noted but more marked. The epithelial cells are reduced to fine granules whilst the nuclei are swollen and stain feebly. There is some diffuse staining of the villi and their tips are blurred and indistinct as if in a condition of coagulation necrosis. The epithelial lining is desquamating in parts. A few bacteria are seen in the tips of the villi but no general invasion has taken place. No large putrefactive bacteria are seen.

*Large intestine* is in a similar condition.

*Liver* —The pattern appears normal under the low power, but on further magnification considerable granularity and swelling of the liver cells is seen. The nuclei are large, rounded and stain poorly.

*Kidney* shows no increase in the appearances noted after three hours except that the staining reaction is diffused and lacks clarity.

No bacteria were present either in the liver or kidney sections.

*Series IV* (9 hours after death)

*Intestines* —Under the low power the pattern is still unaltered but the villi are swollen and indistinct. On further magnification, the changes are considerable in that the epithelial cells have begun to clear and are swollen and free of granules. The nuclei are swollen, vacuolated and indistinct and have little affinity for basic stains. The epithelial margin of the villi is indistinct and many cells are desquamated or digested. The appearance described as withering of the villi is not seen. A few bacteria are seen within the villi and a few large Gram-positive putrefactive organisms are seen at various levels of the intestinal wall. The muscle cells are diffused and stain irregularly, whilst the nuclei are swollen and ill-defined in outline.

The cells of the supra-renal in the lower part of the cortex were swollen and vacuolated as if due to toxic change

No excess of pigment was present

In films of the yellow marrow no cellular element other than fat cells were seen

#### ALIMENTARY CANAL

In the tongue the epithelium was thinned in places and in others shows irritative downgrowth. No monilia was seen.

In the œsophagus the epithelial layers appear thin but are otherwise normal.

The duodenum shows a few areas where villi are withered and atrophic but no loss of epithelium was noted. A small polypoid sessile nodule in the small intestine was found to be an adenoma of Lieberkühn's glands.

The ileum showed mild degenerative changes in the more superficial layers of the mucosa. A good many bacilli resembling *B. welchii* and some cocci invading the superficial layers were observed.

The large intestine shows no abnormality.

#### SUMMARY

The post-mortem appearance differed from those of former sprue cases inasmuch as the emaciation was only of moderate grade and subcutaneous fat was still present. The bone-marrow of the tibia was almost completely aplastic. Atrophic changes in the intestinal mucosa were slight but there were histological appearances in the liver and adrenals indicating the action of a mild toxin.

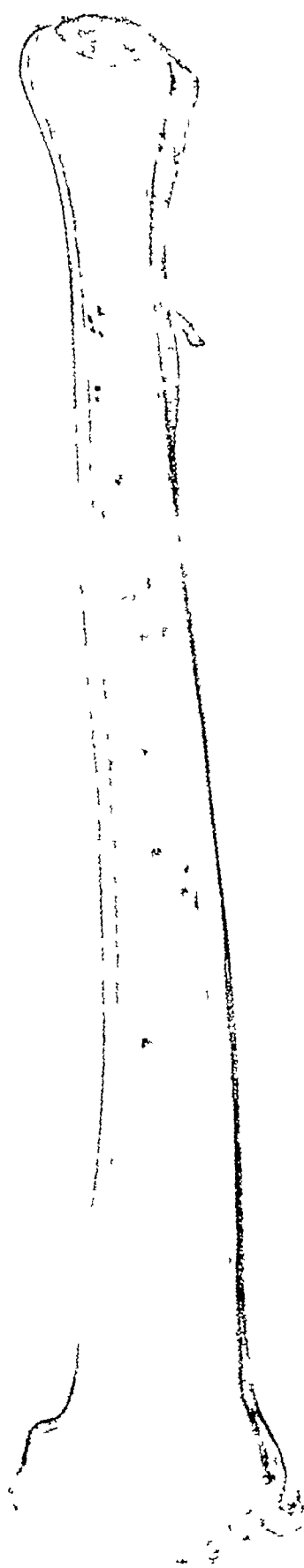


suggesting that they have probably been carried there by the blood stream and not by invasion from a local site after death

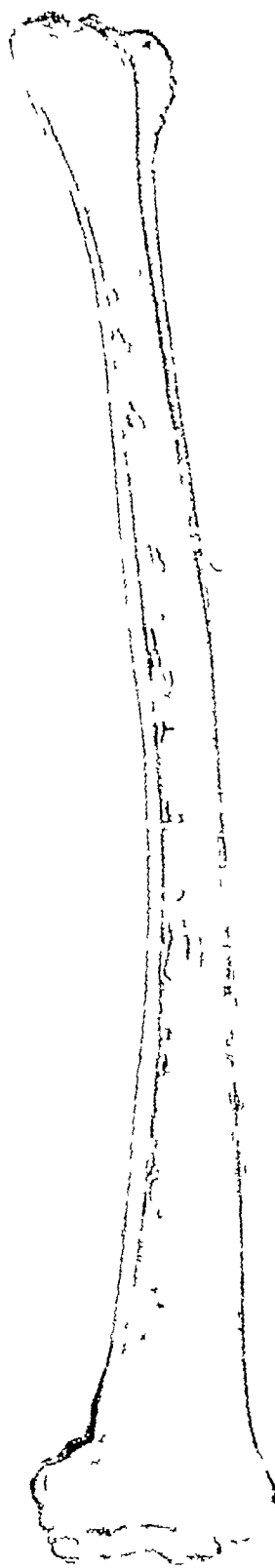
A study of these changes enables one to distinguish those which have been observed in the intestine as a result of disease from those due to putrefaction after death. The description of these changes and the photo-micrographs depicting them may be read in conjunction with the article on the 'Morbidity anatomy of sprue,' which was the object for which these studies in putrefaction were undertaken

#### EXPLANATION OF PLATE LXV

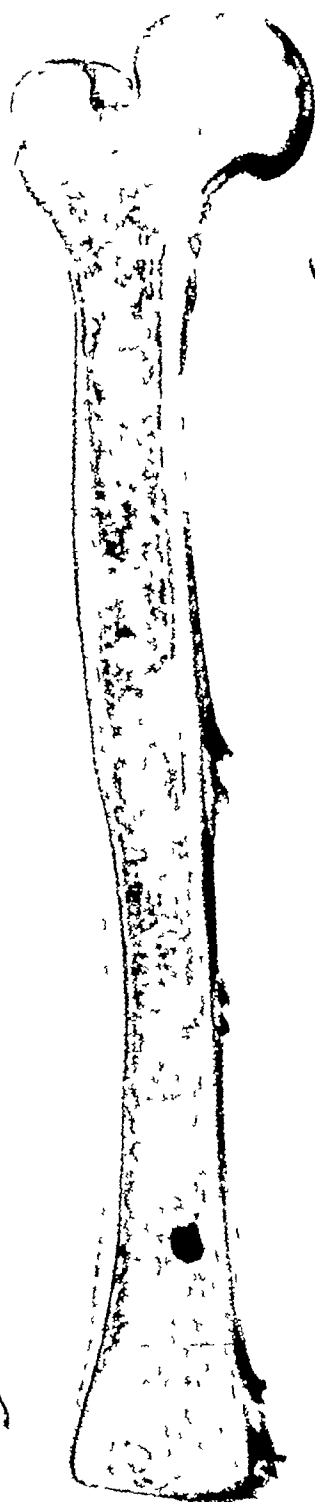
- Fig 1 Small intestine 6 hours after death No visible change under this magnification
- „ 2 Small intestine 9 hours after death The mucous membrane appears clogged and stains diffusely and is lacking in sharpness
- „ 3 Small intestine 9 hours after death Similar changes to those in Fig 2
- „ 4 Large intestine 9 hours after death Coagulation necrosis of the free margin of the mucous membrane, especially in the upper part of the section
- „ 5 Large intestine 9 hours after death Diffused staining and lack of sharpness of histological details
- „ 6 Small intestine 24 hours after death Separation up of the intestinal layer and lack of detail in the various structures  
(Magnification approximately 100)



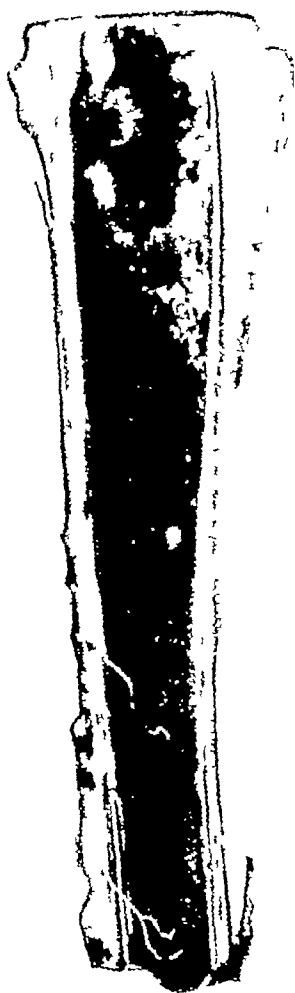
Case No 8



Case No 9



Case No 1



Case No

#### EXPLANATION OF PLATE LXVI

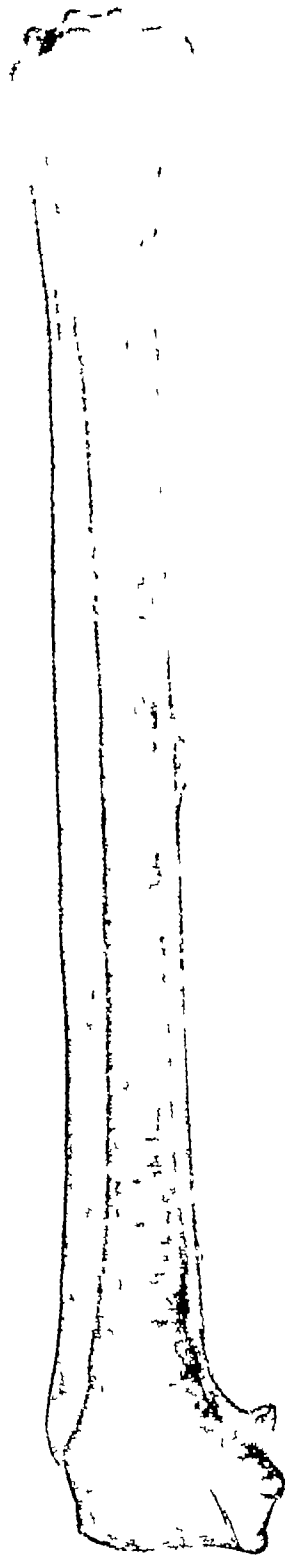
- Fig 1 Small intestine 2 hours after death Shows clogging and loss of detail in the villi
- „ 2 Small intestine 9 hours after death Shows similar changes
- „ 3 Small intestine 9 hours death Still more marked changes resembling coagulation necrosis and diffuse staining
- „ 4 Small intestine 9 hours after death A cross section showing breaking up and vacuolisation of Lieberkuhn's glands
- „ 5 Small intestine 24 hours after death Partial dissolution and clogging up of villi
- „ 6 Longitudinal section of Lieberkuhn's glands Showing vacuolisation and disappearance of cytological details (24 hours after death)
- (Magnification approximately 450 )



Case No 5



Case No 6



Case No 7



Case No 4

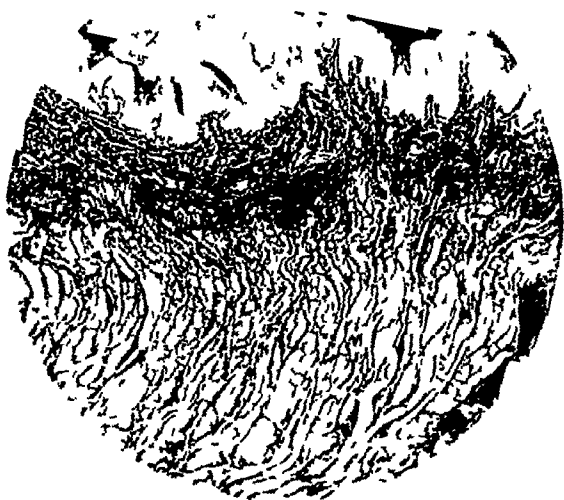
#### EXPLANATION OF PLATE LXVII

- Fig 1 Large intestine 24 hours after death Breaking up and digestion of free surface mucous membrane
- „ 2 Large intestine 24 hours after death Shows a cross section of Lieberkuhn's glands reduced to cell outlines without differentiation of cytological details
- „ 3 Large intestine 24 hours after death Longitudinal section of Lieberkuhn's glands showing similar changes to those in previous figure
- „ 4 Collecting tubules of kidney 24 hours after death Showing diffused staining and lack of finer detail
- „ 5 Cortex of kidney showing similar changes but more advanced (24 hours after death)
- „ 6 Changes of a similar nature in the liver 24 hours after death  
(Magnification approximately 450 )

PLATE LX



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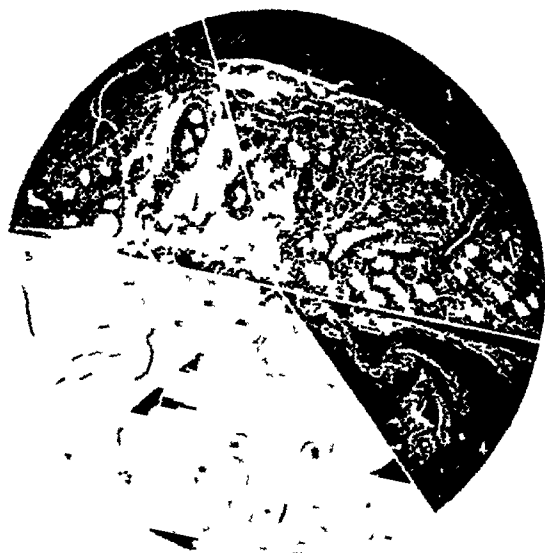
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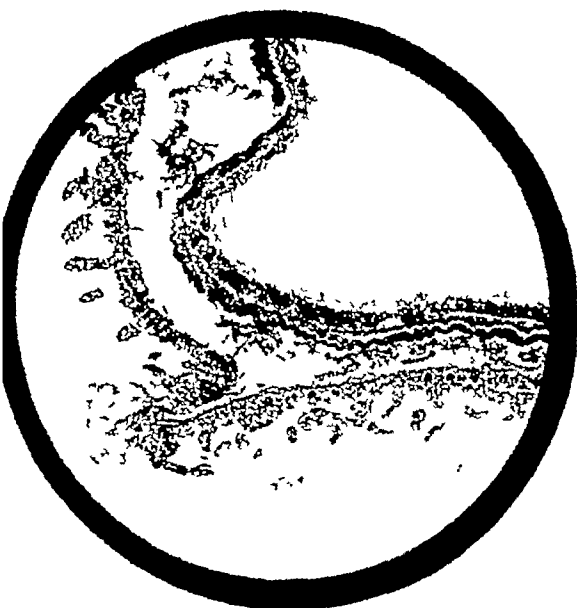




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differential diagnosis from pernicious anæmia may present considerable difficulty and entail detailed laboratory investigations in its elucidation

Thin (1897) described a grave anæmia associated with a high colour index in cases of sprue Bassett Smith (1903) recorded cases of very severe anæmia resembling pernicious anæmia in many respects, but differing in the absence of megaloblasts and the type of colour index Richartz (1905) and Van der Scheer (1905) also recounted examples of this condition, the former observer citing an instance of intense anæmia where the red corpuscles numbered 960,000 per cmm and the hæmoglobin equalled 20 per cent Carnegie Brown (1908) opined that the anæmia was secondary to the absorption of some alimentary toxin which destroyed the circulating erythrocytes In his series red corpuscles were between two and three million per cmm and the colour index was high Poikilocytosis and nucleated cells were absent Begg and Bullmore (1912) reported varying degrees of anæmia, the average red count being 4,170,000, the hæmoglobin 76 per cent, and the colour index varying being sometimes above and sometimes below normal The average white cell count was 5,780 per cmm, while the differential count showed a relative and absolute decrease in the polymorphonuclears, a relative increase in the lymphocytes and a relative and actual increase in the large mononuclears Bullmore regarded the anæmia as similar to that due to pure malnutrition Low (1912) described the different phases of anæmia met with in sprue, hæmatological changes not being in evidence during the early stages, but being characterized by a typical secondary anæmia later Further, progress led to the appearance of poikilocytosis, anisocytosis, basophile degeneration and polychromatophilia, while occasional normoblasts were encountered The terminal picture resembled that of pernicious anæmia In Ceylon, Manson-Bahr (1915) made a detailed study of the blood in his cases The results confirmed the findings of previous investigators and special stress was laid on the pronounced anæmias characterized by a high colour index Changes in the shape and size of corpuscles and polychromatophilia were features of the blood picture, but nucleated reds were scanty The marrow of the sternum was dark red in colour and showed atrophy of the fat cells, but no increase in the erythroblastic elements was noted No evidence was found in the two cases examined at autopsy of any specific affection of the blood forming tissues in sprue, such as is known to occur in pernicious anæmia In this and subsequent work (1923) the opinion was expressed that the anæmia was not due to a primary blood infection, but rather to the constant absorption of some blood destroying toxin from the alimentary canal In the advanced stages an aplastic type of pernicious anæmia was described characterized by an absence of sustained erythroblastic response and of nucleated reds in the peripheral blood Sections and other preparations of the red bone-marrow are stated to show no evidence of an erythroblastic response such as is described in pernicious anæmia

Wood (1919) discussed in some detail the relationship of the two diseases, pointing out that in a large number of cases in the literature the colour index exceeded one In his case no nucleated red cells were present and the fragility test was normal

Rogers (1921) stated that anæmia is an important symptom in the later stages of sprue, being always well marked in fatal cases, usually it is of the secondary type but occasionally manifests a progressive pernicious nature with high hæmoglobin value, in which case the course of the disease is very rapid Out of 28 cases of under one year's duration anæmia was noted in 15, being of high grade in two instances while among 15 cases of over one year's duration it was present in 8 and marked in two Tidy (1923) described the blood changes as being indistinguishable from pernicious anæmia in certain cases of sprue in which recovery occurred, and also drew attention to the fact that little work had been done on achlorhydria in the latter condition

L. W. Smith (1924) refers to a group of cases in which the anæmia of sprue resembled pernicious anæmia in severity and rapidity of course, but with few of the morphological changes characteristic of the former condition The red cell count was under 1,000,000 per cmm and the colour index over one There was a leucopænia and decrease in platelets



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course, though the latter is more typical of sprue. Muscular weakness, sore tongue, anaemia, dyspepsia, diarrhoea, pigmentation, low blood pressure, and extreme asthenia occur in both diseases, and as already indicated the hæmatological findings may not enable a diagnosis to be made.

The diagnostic value of the different clinical findings in these diseases have recently received special attention at the hands of Fairley and Mackie (1926) and Newham, Morris and Manson-Bahr (1926), so that only the more salient and most important features will now be considered.

Pernicious anaemia is rare before the age of 35 years, whereas sprue is commonly met with from 20 years onwards. Marked loss of weight is a prominent and constant feature of the latter disease, the alimentary features of which are generally characteristic. Apyrexial diarrhoea may occur in both classes of patients. The typical stools of sprue, however, are bulky, gaseous and pale coloured and tend to be passed in the early morning hours, while bouts of abdominal distension are almost pathognomonic. The latter come on toward evening and are specially related to fatigue and the carbohydrate moiety of the diet.

The features of postero-lateral sclerosis so commonly observed in pernicious anaemia are rarely if ever met with in sprue, while we have found that the anaemia itself is rarely so advanced when the patient seeks medical advice. Cabot (1927) reviewing a large series of cases of pernicious anaemia states that 84 to 89 per cent of the patients when first seen had blood counts of under 2,000,000 cells per c mm. In his series of 920 cases 681 or 74 per cent showed a colour index of 1 or more, while figures of less than 0.7 were not observed. During the worst phases of the disease the high colour indices were found in 88 per cent, but in the remission period only in 49 per cent, i.e., 51 per cent of cases presented a colour index below unity. Very similar blood findings are recorded in sprue.

Hæmolytic is more marked in pernicious anaemia. This is shown by the lemon yellow tinting of the skin, the highly coloured urine, the golden yellow colour and the increased bilirubin content of the serum, the high pigment content of the duodenal juices as well as the extensive deposits of hæmosiderin found in the liver, spleen, kidney and bone-marrow at autopsy. In contra-distinction to these findings are the dry wrinkled, pale or earthy coloured skin, the lower Van den Bergh readings, and the lesser grade of hæmosiderosis observed in sprue.

Perhaps, however, the most constant feature of pernicious anaemia is achlorhydria and in our hands the fractional test meal has proved a procedure of the utmost diagnostic utility. In a consecutive series of 26 cases reported by Fairley, Mackie and Malandkar (1926), 19 contained free HCl in the gastric content. Of these 4 were hyperchlorhydric, 7 were normal, 8 were hypochlorhydric, while the remaining 7 were achlorhydric in type. In the latter group the total and inorganic chloride curves approximated closely, a finding which suggested that even here HCl was still being secreted, its absence depending on subsequent neutralization by alkaline fluids from the duodenum. With pernicious anaemia,



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blood smears, both L. W. Smith (1919) and Archibald (1919) report an intermediate group of cases characterized by a moderate degree of hæmolysis associated with a failure to produce blood cells. The subjects of pernicious and aplastic anæmia have of necessity been considered at some length for without an adequate appreciation of their clinical, post-mortem and hæmatological features, it is impossible to interpret the pathological findings and the varied blood state observed in sprue.

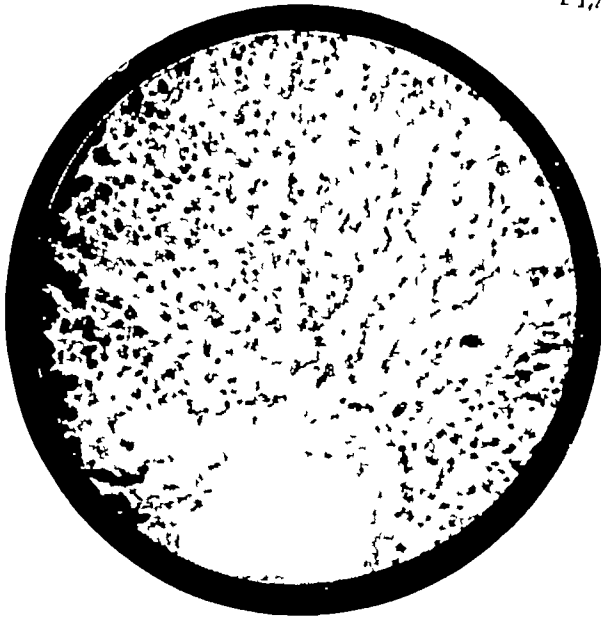
### III THE RED BLOOD CORPUSCLES, HÆMOGLOBIN, COLOUR INDEX AND BLOOD PICTURE

The present series comprises an analysis of the hæmatological data derived from 67 cases of sprue, and the results are recorded in Protocol I. When more than one blood examination was made on the same case, only the earliest estimation was utilized in calculating the mean result for the series.

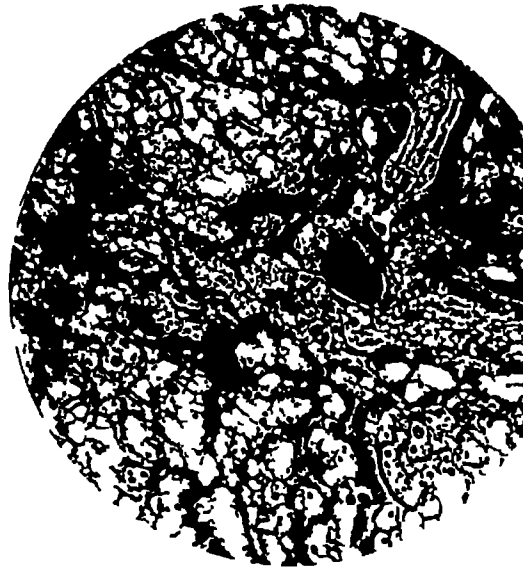
#### PROTOCOL I

*Red blood counts, hæmoglobin percentage, and the Van den Bergh reactions in 67 cases of sprue*

Case No	Age	Sex	Duration of disease	Red blood corpuscles per cmm	Percentage of Hæmoglobin	Colour index.	Indirect Van den Bergh reaction
	27	M	2 weeks	4,900,000	75	0.77	
S 37	34	M	2 weeks	5,500,000	90	0.82	0.4
S 44							
S 100	29	M	3 weeks	5,100,000	109	1.06	0.7
S 81	47	F	1 month	4,450,000	70	0.79	0.4
S 94	45	F	1 month	4,375,000	68	0.78	
S 77	40	M	1½ months	5,300,000	70	0.7	0.5
S 93	37	M	1½ months	5,062,500	85	0.84	0.28
S 25	22	F	2 months	2,050,000	50	1.22	0.6
S 34	40	F	2 months	3,950,000	60	0.76	0.4
S 38	38	F	2 months	4,390,000	64	0.73	0.4
S 42	42	M	2 months	3,810,000	95	1.25	0.6
S 70	19	M	2 months	4,333,333	75	0.86	0.4
S 85	59	F	2 months	2,875,000	60	1.04	1.1
S 92	48	F	2 months	4,000,000	95	1.1	0.52
S 103	27	F	2½ months	4,875,000	97.5	1.0	0.4
S 101	29	M	Over 2 months	2,537,000	63	1.24	



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3



4



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PROTOCOL I—*contd*

Case No	Age	Sex	Duration of disease	Red blood corpuscles per cmm	Percentage of Haemoglobin	Colour index	Indirect Van den Bergh reaction
S 3	42	M	12 months	2,600,000	50	0·96	
S 86	30	M	12 months	3,225,000	55	0·85	0·6
S 89	33	M	12 months	1,100,000	40	1·82	1·1
S 95	45	F	12 months	3,075,000	70	1·12	0·6
S 6	20	M	1 year 3 months	2,850,000	87	1·52	
S 33A	54	M	1 year 3 months	3,350 000	70	1·04	0·3
S 5	65	M	1 year 6 months	4,800,000	78	0·81	
S 29	27	M	1 year 6 months	4,060,000	80	1·0	0·6
S 98	47	F	1 year 6 months	1,462 500	35	1·19	
S 8	30	M	1 year 7 months	2,400,000	60	1·25	
S 23	47	F	1 year 8 months	2,800,000	70	1·25	1·0
				3,066,666	60	0·98	
S 27	37	F	1 year 9 months	2,780 000	48	0·86	0·4
			1 year 10 months	1,816,000	42	1·16	
			1 year 11 months	1,000,000	20	1·0	0·9
S 33	54	M	1 year 9 months	3,933,333	80	1·02	2·0
S 1	50	M	2 years	3,666,666	65	0·89	
S 20	29	M	2 years	2,850,000	70	1·23	0·25
S 87	40	F	2 years	1,937,500	50	1·3	0·55
S 24	42	F	2 years 2 months	2,450,000	65	1·33	1·2
S 82	27	M	2 years 9 months	1,250,000	30	1·2	0·3
S 84	51	M	3 years	2,400,000	55	1·15	0·4
S 16	35	M	4 years	3,050,000	55	0·90	0·4
S 83	55	M	4 years	3,375,000	65	0·96	1·1
S 97	40	M	4 years	3,187,000	72	1·13	.
S 99	29	F	4 years	2,975,000	49	0·82	
S 79	26	M	5 years	1,493,750	30	1·0	0·4
S 4	31	F	6 years	2,600,000	50	0·96	.
S 26	50	M	Chronic	2,650,000	65	1·23	1·0

# THE MICROSCOPICAL CHANGES OCCURRING IN ORGANS AFTER DEATH

BY

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(Being Part V of the Sprue Investigation at the Haffkine Institute, Bombay)

[Received for publication, April 10, 1928]

IN our study of the changes in the intestine of sprue cases, we realized that some of the appearances we described might be due to, or at least were coincident with, those due to decomposition.

The importance of being able to recognize post-mortem change is a problem which confronts all histologists, but particularly those working in the tropics.

We set ourselves the task of observing such changes in orderly sequence and, as human material is not easy of control, we used the organs of monkeys. A healthy monkey was killed by chloroform and the intestinal canal, liver and kidney were removed *in toto* and kept in sterile dishes. Portions of the upper part of the small intestine, the lower part of the small intestine, of the large intestine, of the liver and kidney were removed as follows and placed in fixative for subsequent section. The intestines were not opened or washed out before fixation.

Series I Killed at 9-30 a.m. (controls)

Series II Taken at 12-30 p.m., i.e., after 3 hours at room temperature (about 70-75°F)

Series III Taken at 3-30 p.m., i.e., six hours after death (3 hours at room temperature and 3 hours in incubator at 98.4°F) \*

Series IV Taken at 6-30 p.m., i.e., nine hours after death (3 hours at 75°F, 6 hours at 98.4°F)

Series V Taken next morning at 9-30 a.m., i.e., 24 hours after death (3 hours at 75°F, 6 hours at 98.4 and 15 hours at room temperature 70-75°F)

Sections were then cut of all these portions and were stained hæmatoxylin and eosin and by Gram's method for bacteria. *Series I* were taken as normals for comparison. The following is a brief résumé of the principal changes observed —

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\* This temperature was taken as representing the maximum diurnal indoor temperature in Bombay during the hot weather.



*(b) The hæmoglobin*

The average hæmoglobin estimation for the series was 65.5 per cent, the minimum and maximum values recorded being 10 per cent and 109 per cent, respectively. As would be expected, the latter result was obtained at the onset of the disease.

The actual findings may be grouped as follows —

1 case or	15	per cent showed less than	20	per cent hæmoglobin
7 cases	10.4	" " " "	40	" "
20	29.8	" " " "	60	" "
49	73.1	" " " "	80	" "
66	98.5	" " " "	100	" "
1 case	15	" exceeded	100	" "

The incidence in the different groups may be studied in Plate LXIX, and an interesting comparison of the results obtained in the case of the red blood corpuscles is afforded. The tendency is for a larger proportion of the cases to fall into the higher groupings in so far as the hæmoglobin is concerned—a finding which is related to the high colour indices which characterize the disease after the first few months of onset.

When the results of the hæmoglobin estimations and the red blood counts are considered conjointly, we see that moderate anæmia is present in the great majority of cases with fully developed sprue, and the severe grades of anæmia are not infrequent. Profound anæmia, on the other hand, with a hæmoglobin value of less than 15 per cent is extremely rare. Further, our results conclusively show that when medical advice is first sought, the majority of sprue cases are suffering from a less severe grade of anæmia than are patients with pernicious anæmia.

*(c) The Colour Index in Sprue*

The colour index is of special importance in any blood investigation as it affords information not only regarding the hæmoglobin-carrying capacity of the corpuscles, but also in classifying the anæmias into the so-called secondary and primary types.

The mean figure obtained for the 67 cases of our series was 1.0, the lowest being 0.7 and the highest 1.8.

The group distribution was as follows —

6 cases gave readings between	0.7 and 0.79
13 " " " "	0.8 " 0.89
7 " " " "	0.9 " 0.99
15 " " " "	1.0 " 1.09
8 " " " "	1.1 " 1.19
12 " " " "	1.2 " 1.29
2 " " " "	1.3 " 1.39
2 " " " "	1.4 " 1.59
2 " " " "	1.7 " 1.89

The *kidney* and *liver* cells showed diffused staining and loss of definition and the cytoplasm was reduced to a mass of ill-stained granules, but the nuclei were still well differentiated. The lobular pattern of the *liver* is lost and the whole parenchyma stains homogeneously. A few small groups of putrefactive bacteria were seen in both these viscera.

#### *Series V* (24 hours after death)

*Intestines*—The general pattern of the various layers is still retained but the tissues stain a brownish red with eosin and the hæmatoxylin stain has taken very little. The layers of the intestinal wall are separating up, and there are some small spaces as if ruptured by gas production. The epithelium of Lieberkuhn's glands is clear and vacuolated and the cells are only represented by the cell wall, the cytoplasm and nuclei having disappeared leaving clear spaces. The villi are clogged and structureless and stain diffusely. The muscle cells are converted into a dirty brown homogeneous layer with a few swollen nuclei here and there. Bacteria of various kinds are seen to be present in the whole gut wall and large putrefactive bacteria are in small groups.

*Liver*—All lobular pattern is lost and the whole parenchyma reduced to a mass of ill-defined and ill-staining polyhedral cells. The nuclei are retained but are swollen, vacuolated and badly stained.

*Kidney*—The general pattern of tubules and glomeruli is retained, but all finer details lost. The structure of the glomeruli has survived better than that of the tubules which are diffused and homogeneous and stain a dirty brownish red with eosin. Groups of putrefactive bacteria are seen scattered throughout the liver and kidney.

#### SUMMARY

The microscopical changes due to putrefaction as studied in the intestines, liver and kidney are discernible three hours after death at room temperature (75°F) and proceed in an orderly manner as far as they have been followed (24 hours). The first changes are discernible in the epithelial cells of the kidney and of the intestine, and are of the nature of granularity of the cytoplasm, swelling and indistinctness of the nuclei and slight changes in tinctorial reaction. As putrefaction proceeds, the epithelial cells and the parenchyma cells of the liver gradually break up, lose their outlines, become hazy and eventually merge into an homogeneous mass. The nuclei first become swollen and vacuolated, lose their affinity for hæmatoxylin and eventually dissolve up. Muscle cells withstand the process longer but eventually show similar changes. The connective tissues retain their characters still longer and for this reason the general architecture of the organ is retained long after the parenchymatous cells have become diffuent or are dissolved.

Intestinal bacteria begin to penetrate the intestinal mucosa about nine hours after death and putrefactive bacteria are met with in increasing numbers after this time, and their distribution is generalized equally throughout the tissues.

## IV THE LEUCOCYTES

In sprue most observers who have investigated the leucocytes have described a condition of leucopænia associated with a relative lymphocytosis

The results obtained in our series are recorded in Protocol II. The average total leucocytes count in 60 cases equalled 6,728 per cmm, while the differential count in 61 patients yielded the following results —

Polymorphonuclear neutrophile leucocytes = 55.5 per cent

Polymorphonuclear eosinophile leucocytes = 1.7 per cent

Lymphocytes = 37.8 per cent

Large mononuclears = 3.3 per cent

Transitional mononuclears = 1.7 per cent

## PROTOCOL II

*The total and differential leucocytic count in 61 cases of sprue*

Case No	Total leucocytes per cmm	DIFFERENTIAL COUNT PERCENTUM				
		Polymorpho-nuclear leuco-cytes	Eosinophile leucocytes	Lympho-cytes	Large mono-nuclears	Transitional mononu-clears
S 1	8,125	55.7	0.4	38.6	1.4	3.9
S 2	3,125	47.4	0.0	51.4	0.8	0.4
S 3	7,188	57.5	0.5	35.5	3.5	3.0
S 4	4,375	43.0	2.0	49.5	3.0	2.5
S 5	5,625	50.0	2.0	40.5	3.0	4.5
S 6	6,875	41.9	2.8	48.4	6.9	
S 8	3,750	64.0	1.0	17.0	13.0	5.0
S 16	4,687	31.9	0.5	64.3	1.4	1.9
S 17	10,000	63.5	3.5	26.5	2.5	4.0
S 18	5,625	39.0	0.5	59.5	1.0	0.0
S 19	6,250	41.7	0.5	50.0	4.4	3.4
S 21	11,250	71.8	0.0	25.2	2.0	1.0
S 22	8,125	54.6	1.1	41.4	2.7	0.2
S 23	3,437	64.4	3.9	28.7	0.0	3.0
S 24	8,750	52.4	2.4	38.7	0.6	5.9
S 25	2,500	62.4	0.2	28.5	8.1	0.8
S 26	3,125	51.4	1.4	30.7	16.3	0.2
S 27	4,687	35.0	0.4	55.0	9.2	0.4



1



2



3



4



5



PROTOCOL II—*concl'd*

Case No	Total leucocytes per c mm	DIFFERENTIAL COUNT PERCENTUM				
		Polymorpho-nuclear leuco-cytes	Eosinophile leucocytes	Lympho-cytes	Large mono-nuclears	Transitional mononu-clears
S 86	4,375	49.4	1.0	48.4	0.8	0.4
* S 87	5,000	74.4	0.4	21.8	1.6	1.4
S 88	7,500	58.8	0.6	37.6	1.0	2.0
S 89	5,625	60.2	1.8	34.8	1.6	1.6
S 90	3,750	38.0	1.2	58.6	0.4	1.8
S 91	12,500	71.0	1.4	25.0	1.3	1.3
* S 92	5,000	63.2	1.6	30.6	1.6	2.8
S 93	6,250	77.2	2.0	16.2	1.8	2.8
S 94	6,250	50.2	1.0	44.6	1.8	2.4
S 95	5,000	63.3	1.0	30.3	3.1	2.3
S 96	3,125	60.3	1.0	33.3	4.0	1.4
S 97	5,000	53.2	1.0	43.6	0.6	1.6
S 98		31.0	1.0	65.2	1.2	1.6
S 99	2,500	32.2	1.2	63.6	1.6	1.4
S 100	7,500	56.2	2.0	39.2	1.2	1.4
S 101	2,500	49.2	19.8	26.6	2.4	2.0
S 103	12,500	72.3	2.6	21.1	3.0	1.0
Average count	6,728	55.5	1.7	37.8	3.3	1.7

\* Basophile leucocytes equalled 0.4 per cent in S 87 and 0.2 per cent in S 92

The mean of the total series thus falls within normal limits, but when the results are analysed in more detail it is seen that three groups of cases occur, and these are tabulated in a separate protocol III. It is here shown that 33 out of 60 or 55 per cent show a total of under 6,000 leucocytes per c mm, 22 or 36.6 per cent yield readings varying from 6,000 to 11,900 per c mm, while a smaller series of 5 cases have counts falling into a 12,000—21,000 group. Of these probably only the intermediate cases of Group II fall within physiological limits, those in Group I, showing a leucopænia, and those in Group III a leucocytosis.

The differential counts in Groups I and II tend to show an increase in the lymphocytes at the expense of the neutrophile granular leucocytes, but many of the findings cannot be regarded as abnormal. In the case of Group III, however,



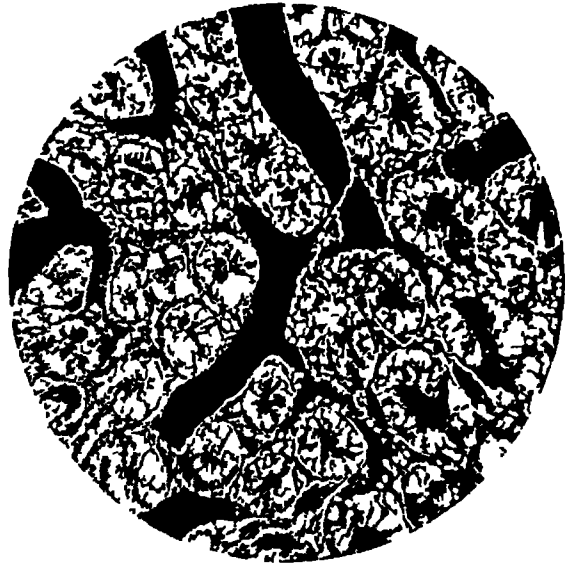
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3



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5



6

of whitish semi-solid stools, loss of weight, asthenia and weakness. She was treated for sprue, gained 10½ lbs in weight and was discharged as cured on the 8th September, 1923.

On the 21st May, 1925, some 21 months later she was re-admitted to hospital complaining of a sore tongue, morning diarrhœa, and loss of weight. She dated the onset of the present attack to 4 months previously when after eating ox-tail soup she developed a sore tongue and severe nausea and vomiting which lasted for a week. Diarrhœa followed and this had persisted on and off ever since. She also complained of cramp in the limbs and abdominal distension after food. Hot fluids and spiced foods could not be tolerated. On examination the patient was emaciated and anæmic. The tongue had recently been very sore with ulcers and fissuring, and though the more acute inflammation was subsiding, red granulations were present at the tip and edges. The papillæ were prominent laterally, but considerable atrophy had occurred on the dorsum where the mucous membrane was smooth and atrophic. The teeth were carious and pyorrhœa was marked. The blood pressure was 110/76, and the motions on admission were small, brown and watery. The fasting content as well as the fractional test meal showed marked hyper-acidity, a reading of 70 c.c.s. N/10 per cent being obtained at the end of 3½ hours. Starch disappeared in 2½ hours. Two different estimations of the ionic calcium by Vines's method showed values of 7.4 and 7.6 mg per 100 c.cm.

On the 21st May, 1925, the Van den Bergh reaction = 0.4 units, while the red blood corpuscles = 2,780,000 per c.mm., the hæmoglobin = 48 per cent and the colour index 0.86.

The patient was given a full course of Scott's treatment of calcium lactate and parathyroid. Unfortunately, though she did not lose weight she became progressively weaker and failed to respond satisfactorily.

On the 6th June, 1928, she developed a temperature for 2 days and on the 13th June, 1928, i.e., 23 days later, blood examination revealed a big fall in the red blood corpuscles which now equalled 1,816,666 per c.mm. The hæmoglobin = 42 per cent, and the colour index 1.17. Subsequently the diarrhœa increased. The patient now developed a suppurative gingivitis and ran a continuous temperature for 9 days.

On the 13th July, 1925—one month later—the red blood corpuscles = 1,000,000 per c.mm., the hæmoglobin 20 per cent, and the colour index = 1. An indirect Van den Bergh reading of 0.9 units was obtained. The patient now began to develop fainting attacks, asthenia and breathlessness were marked, and she died of profound anæmia on the 2nd August, 1925.

Special features of interest in this case are the rapid progression of the anæmia despite milk, calcium and parathyroid treatment, the low indirect Van den Bergh readings, and the absence of stigmata of regeneration in the peripheral blood.

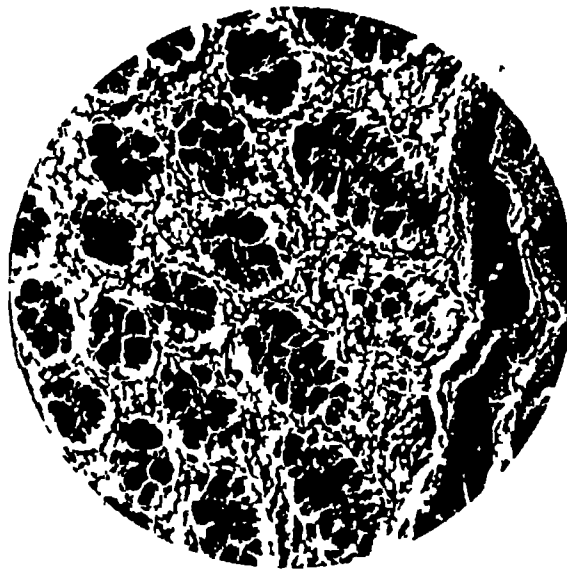
The two Van den Bergh readings taken at an interval of 7 weeks (0.4 and 0.9 units respectively) indicate that no hæmolytic factor underlay the anæmia, while the absence of nucleated red cells in the blood smears supports the view that the fault lay primarily with the bone-marrow which, poisoned by the sprue virus and poorly nourished as a result of semi-starvation, had proved utterly incapable of an adequate and sustained erythropoietic response. Though no autopsy was available in this particular instance, it is highly probable that the onset of the crisis was determined by the transformation of a hyperplasia of the red marrow into one of aplasia—a state of affairs in accord with our other post-mortem findings.

## VI THE PRICE-JONES CURVES IN SPRUE

Price-Jones (1920) utilizing dry stained blood films described a method of accurately measuring the diameter of the red blood corpuscles and classifying them



1



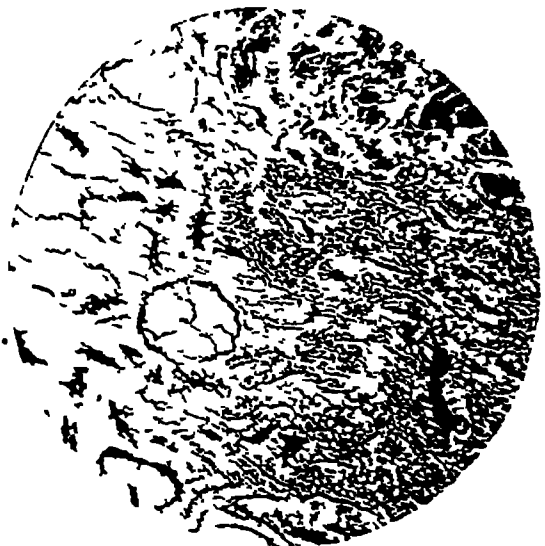
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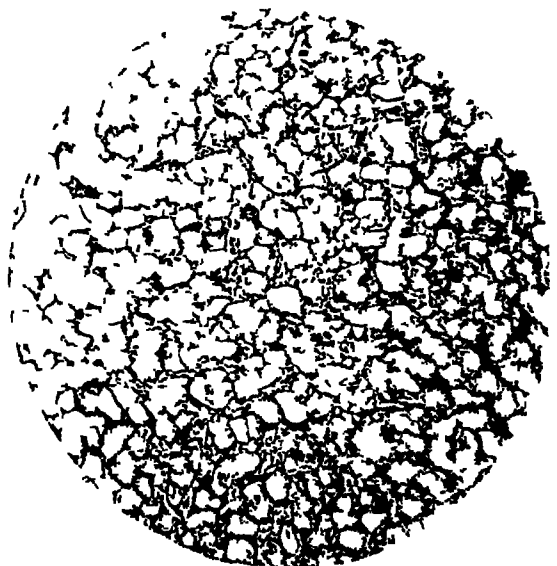
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many of the hæmatological features of pernicious anæmia including a typical Price-Jones curve, an aplastic condition of the marrow of the tibia was found at autopsy.

As a rule the curves were extremely asymmetrical and their bases broadened. This widening invariably extended to the right and in several of the cases to a lesser degree to the left as well. Anisocytosis in sprue may, therefore, occur in two directions, but as the macrocytes and megalocytes vastly outnumber the microcytes the net result is a definite increase in the average size of the red cells. In some instances microcytes are absent from the blood films.

In our series no correlation was found to exist between the percentage of hæmoglobin and mean diameter of the red cells and it is interesting to note that in the only case (No. 99) which a colour index of less than 1, the mean diameter of the corpuscles was  $7.74 \mu$ , the peak of the curve was markedly displaced to the right and the base was broadened in this direction also.

In our experience the Price-Jones curves in cases of sprue with well established anæmia show displacement to the right of the normal, broadening of the bases and marked asymmetry, and in all respects, including the increased mean diameter of the red blood corpuscles, closely resemble those obtained in pernicious anæmia. Our findings support those of Hampson and Shackle (1924) that sprue is essentially a megalocytic anæmia, and the results of the Van den Bergh test in several of our cases conclusively show that blood may be megalocytic in type even in the absence of any evidence of increased hæmolysis, i.e., of hyperbilirubinæmia.

## VII THE VAN DEN BERGH REACTION

In view of the fact that the Van den Bergh reaction affords information regarding the hæmolytic basis of a given anæmia, the results obtained by this test in sprue have received special consideration during the present investigation.

Van der Scheer (1924) recorded increased quantities of bilirubin in the blood of sprue cases and sometimes of urobilin in the fæces, but in his opinion the blood destruction never reached a degree comparable to that observed in pernicious anæmia. Hampson and Shackle (1924) reported indirect positive Van den Bergh reactions in 3 cases of sprue, and did also Newham, Morris and Manson-Bahr (1926) in 6 out of 7 cases in their series, but the number of Van den Bergh units were not given in either communication. Fairley, Mackie and Malandkar (1926) reported a consecutive series of 16 cases of sprue, 9 giving readings under 0.5 units and 7 above this number. Only one in the latter group exceeded 1.3 units, and in this instance a reading of 2.0 units was obtained. They considered the reaction afforded valuable information in the differential diagnosis of sprue and pernicious anæmia. Sokhey, Malandkar and his colleagues (1928) reported a series of 13 cases, seven of which showed not more than 0.6 units of bilirubin in the serum, and 4 not more than 1.1 units. In the other two, 3.0 and 4.0 units were recorded. They did not, however, regard the reaction as affording information of value in distinguishing between the two conditions.

Morris (1926) estimated the bile pigment products by the method of Terwen in 3 cases of sprue, and found that in the two patients with severe anæmia the average daily output of urobilin in the urine and fæces was much above normal, but that in the third who did not suffer from marked anæmia the output was within normal limits. The index of blood destruction according to Lichtenstein's standard was 4.9, 2.7 and 1.4 times as rapid as normal.

# ANÆMIA IN SPRUE

## AN ANALYSIS OF 67 CASES

BY

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*(Being Part X of the Sprue researches at the Haffkine Institute)*

[Received for publication, October 10, 1928]

THE condition of the blood in sprue has constituted a problem of continuous difficulty to physicians investigating tropical disease during the last three decades, and for four years various members of the staff of this Institute have been engaged in collecting hæmatological data from patients admitted to the wards of St George's Hospital, Bombay, suffering from this disease

In addition to the routine hæmatological investigations the Van den Bergh reaction has been employed to estimate the bilirubin content of the serum and special efforts have been made to determine the hæmolytic or non-hæmolytic nature of the anæmia Price-Jones curves were obtained in 11 typical cases in order to ascertain whether the anæmia was of megalocytic type and whenever possible the condition of the bone-marrow was correlated with the hæmatological findings in the peripheral blood

### I PERNICIOUS ANÆMIA AND SPRUE

As a general rule in early cases met with in the tropics, the anæmia is not far advanced and the lingual and gastro-intestinal features are sufficiently typical to permit a diagnosis to be made on clinical grounds In patients seen for the first time at a later date the anæmia may constitute a dominant feature of the clinical picture, and if the alimentary features are atypical or in abeyance, the

100 c cs (i e, 1.5 units) Green and his co-workers at the Mayo clinic, taking into account cases of familial cholæmia or physiological hyperbilirubinæmia estimate it at 2 mg per 100 c cs or 4 units Schiff (1927) has recently examined a large series of normals and he estimates the upper limit of normality as 1 mg per 100 c cs (i e, 2 units), while values above 0.6 mg per 100 c cs (i e, 1.2 units) may indicate a pathological increase In his series 63.4 per cent of cases showed readings below 0.3 mg per 100 c cs (i e, 0.6 unit) and 89 per cent were below 0.5 mg (i e, 1.0 unit)

### *Analysis of the Results*

The sera of 48 of our cases were examined for bilirubinæmia by the biochemical unit of the Institute Of these the first 25 were tested by Malandkar utilising the technique advocated by McNee (1923), while the second 23 were investigated by Sokhey and his colleagues adopting the improved modification of McNee and Keefer (1925) but not employing caffeine sodium salicylate as its effect was found inconstant A close parallelism was observed between the two series of results which are detailed in Protocol I

The average of the 48 cases equalled 0.33 mg per 100 c cs or 0.66 unit, and briefly epitomized the findings were as follows —

- Group (1) 26 cases (54.17 per cent) were under 0.6 unit
- „ (2) 35 cases (72.9 per cent) were under 0.8 unit
- „ (3) 44 cases (91.7 per cent) were under 1.2 units
- „ (4) 4 cases (8.3 per cent) varied between 1.2 and 3.0 units

In the last group three with readings of 1.2, 2.0 and 3.0 units were suffering from intercurrent malaria, while in the fourth (1.2 units) a history of recent malaria was obtained Kingsbury (1926) showed that high bilirubin values are obtained in malignant tertian malaria Ross (1927) confirmed these results and in a series of 29 cases where parasites were demonstrable recorded indirect readings of under 1.0 unit in only four cases, the remaining twenty-five yielding results varying from 1.0 to 4.8 units In view of such findings we do not feel justified in attributing these higher readings of from 1.2 to 3.0 units exclusively to sprue

Of the remaining 44 cases 30 or 68.2 per cent according to McNee and 36 or 81.8 per cent according to Fishberg's standard would be classified within normal limits Schiff on the other hand would consider that they all fall into this category

A clearer presentation of the facts may be obtained by a perusal of Plate LXXII Here the findings in our series of 48 sprue cases are contrasted with those reported by Schiff in 202 healthy individuals, by Ross in 29 cases of malaria showing M. T. parasites in the peripheral blood and by Andrewes and Fishberg in a composite group of 27 patients suffering from pernicious anæmia and investigated during the more active phases of that disease

Nucleated red cells and polychromatophilia were absent. The condition was one of idiopathic aplastic anaemia.

Byrom Bramwell (1924) recorded his impressions of the similarity between certain cases of sprue and pernicious anaemia, and suggested that parathyroid therapy was worthy of trial in the latter disease. Van der Scheer (1924) pointed out that the microscopical constitution of the blood sometimes greatly resembles pernicious anaemia and that accelerated blood destruction was indicated by the increased quantities of bilirubin in the blood, and sometimes of urobilin in the faeces. This destruction, however, never reached a degree comparable to that observed in pernicious anaemia. The haemoglobin metabolism, as estimated by the bilirubin of the blood serum and the urobilin of the excreta, is stated to afford an index to improvement or otherwise of the patient. In one case the daily average bilirubin was 0.322 gm. Elders (1925), who is convinced that sprue is a deficiency disease, cites instances in which the blood picture cannot very well be distinguished from pernicious anaemia and states that tongue changes and achlorhydria may be characteristic of both conditions. In one case of sprue, bilateral ataxia and anaesthesia of the legs were present. Hunter (1925) criticized Elder's calculations of colour index on the basis that his figures were higher than they should be. Culland and Goodall (1925) recorded that they had seen cases of sprue which were undoubtedly examples of pernicious anaemia. They give cell counts on three cases with high colour index which resembled the blood picture of pernicious anaemia in all respects except that nucleated red cells were absent. In a later case prior to death they found the RBC's = 780,000 per cmm and the haemoglobin 20 per cent. Numerous megaloblasts were present. In a case successfully treated with parathyroid and calcium lactate, the colour index was observed to fall as the patient improved.

Fairley, Mackie and their colleagues (1925) in a progress report on researches in sprue found that in a series of 25 cases, the red blood corpuscles averaged 3,243,490 per cmm, the haemoglobin 65.1 per cent and the colour index 1.0. The average total leucocyte count equalled 6,376 cells per cmm, while the differential white count showed a relative decrease in the polymorphonuclear neutrophile leucocytes and an increase in the lymphocytes. In blood smears poikilocytosis, anisocytosis or polychromatophilia were noted, but nucleated red cells, especially megaloblasts, were rarely seen.

Newham, Morris and Manson-Bahr (1926) in their special study of sprue and Addisonian anaemia published a detailed protocol of 12 cases, ten of which presented a colour index exceeding unity. The red cells varied between 1,000,000-4,000,000 per cmm, and leucopenia with a relative lymphocytosis constituted a frequent finding. Anisocytosis and poikilocytosis were commonly observed and from 1 to 3 nucleated red cells were generally demonstrated for each 500 leucocytes counted.

Fairley (1927) reviewed the chief features found of assistance in Bombay in differentiating sprue and pernicious anaemia emphasizing the importance of the Van den Bergh reaction and of the fractional test meal. Baumgartner and Smith (1927) dealt with and supposed identity of sprue and pernicious anaemia. In their series of 15 cases three had achylia, and in four the red cell count was less than 200,000, and in 9 the colour index exceeded unity. Eight showed a diminution of leucocytes and blood platelets. The authors believe that the anaemia is aplastic in type.

Piney (1927) in his recent work on haematology states that sprue is due to the fungus *Monilia albicans* and that certain resemblances exist between it and pernicious anaemia but that unfortunately no details are available. The above epitomy of the literature hardly supports this contention, though we agree that the diversity of the blood conditions described by different observers in sprue has in the past led to much confusion. It was with the object of clarifying our own and other writers' views on this subject that the present investigation was undertaken.

From the clinical viewpoint also sprue and pernicious anaemia have certain common features. Each may be characterized by remissions and an afebrile

J M, an Englishman, aged 62 years, was first admitted to St George's Hospital with an afebrile diarrhoea and malaria (B T) on the 6th August, 1923, and subsequently in October and December of the same year for relapsing sprue.

On the 12th April, 1924, he returned with another relapse, the intestinal and lingual features of which were typical. At this time the red cells equalled 4,000,000 per cmm, the hæmoglobin was 70 per cent, and the colour index 0.88. The leucocytes were 6,250 per cmm. The blood films showed some megalocytes but no nucleated red cells. Some poikilocytosis and polychromatophilia were present. After appropriate treatment his condition greatly improved and he was duly discharged from hospital.

On the 3rd August, 1924, he was re-admitted in a collapsed condition. The tongue was inflamed and the stools which were mainly passed in the early morning hours were large, pale, foul smelling and frothy. Blood counts were made on the 8th August, 1924, and they showed that a marked increase in the anæmia had occurred during the intervening 4 months. The red blood corpuscles were now 2,400,000 per cmm, the hæmoglobin 45 per cent, and the colour index 0.94. The leucocytes numbered 5,625 per cmm. Blood smears showed some poikilocytosis, and polychromatophilia and basophilic degeneration were also noted as well as two erythroblasts. Death occurred on the 13th August, 1924.

At autopsy the spleen appeared normal but soft red 'raspberry jam' marrow filled the entire medullary cavity as well as all spaces in the cancellous bone of the tibia. Smears of the bone-marrow showed megaloblasts and nucleated red cells in quantity. The so-called 'megaloblastic degeneration of Ehrlich' was present and the macroscopic features which may be studied in Coloured Plate LVIII, No. 3, appeared identical with those observed in pernicious anæmia.

The feature of interest here is the occurrence of bone-marrow changes in a typical case of sprue similar to or identical with those observed in pernicious anæmia. The hæmatological findings also resemble those seen in the latter disease, and though the colour index does not exceed unity, it is sufficiently close (0.94) to receive no special comment.

The next case No. 80 has already been referred to in the section dealing with the Price-Jones curves.

S. 80 D. M., a Scotchman, aged 32 years, was admitted to St George's Hospital, Bombay, on the 16th February, 1927, with diarrhoea of 12 days' duration. For several years he had been troubled with soreness of the tongue and the clinical picture in regard to both the tongue and the stools was typical of sprue. Severe emaciation and anæmia were present, and a history of dyspepsia was obtained. The spleen was definitely enlarged and there was a history of recurrent malaria for the past two years.

The red blood corpuscles numbered 1,762,000 per cmm, the hæmoglobin was 40 per cent, and the colour index equalled 1.1. The total leucocyte count was 4,375 per cmm and the indirect Van den Bergh test definitely positive (3.0 units). Films showed anisocytosis, a few microcytes and poikilocytes but no normoblasts. The Price-Jones curve (Plate LXX, No. 80) was displaced to the left, the base was broadened while the average mean diameter of the corpuscles equalled  $7.11 \mu$ . The patient left hospital against advice on the 23rd February, 1927, and was re-admitted on the 10th June, 1927, almost moribund and profoundly anæmic.

Examination of the blood six days before death, i.e., 128 days after the preceding hæmatological examination showed the progressive nature of the anæmia for now the red blood corpuscles equalled only 400,000 per cmm, the hæmoglobin 10 per cent, and the colour index 1.25. The leucocytes numbered 2,812 per cmm. The indirect Van den Bergh reaction now gave a reading of only 2.0 units.

The blood itself was thin and watery and left no visible film on the slide. In size the corpuscles were remarkably constant and only a few megalocytes and poikilocytes were seen.

on the other hand, the achlorhydria is a true *achylia gastrica* due to a primary secretory defect. Such findings conclusively show that sprue and Addisonian anæmia are distinct clinical entities.

## II APLASTIC ANÆMIA AND SPRUE

A few writers, including Manson-Bahr (1923), Smith (1924) and others have noted the occurrence of an aplastic type of anæmia in sprue, but owing to the rarity of aplastic anæmia in clinical practice and the lack of examination of the bone-marrow at sprue autopsies, the relationship existing between the two diseases has never been adequately studied.

The first case of aplastic anæmia was reported by Ehrlich (1888) and since that date numerous writers, notably Cabot (1908), Schneider (1918) and Sheard (1924) have analysed the recorded cases and reviewed the literature. A total of some 130 have been reported. Aplastic anæmia is generally attributed to a grave failure on the part of the bone-marrow to manufacture erythrocytes, granular leucocytes and platelets. It is considered to result from some bacterial toxin or other noxious agent like benzol, T N T, radium or X-rays causing a degeneration or disappearance of the marrow elements. The condition may also develop as a terminal aplasia in pernicious anæmia, and as is recorded later a similar transition occurs in sprue also. Hayes-Smith (1928) as a result of careful observations on a single case of aplastic anæmia treated with liver substance (Armour) has suggested that the liver is the organ primarily involved, the aplasia of the marrow resulting from deficient production of some hepatic hormone which is essential for its normal functioning. Clinically aplastic anæmia is characterized by a rapid, non-remittent course of from a few weeks to 15 months duration, and is associated with fever, hæmorrhages into the skin and mucous membranes, and the classical features of a profound anæmia.

Blood examination shows marked decrease in erythrocytes, granular leucocytes and blood platelets, while regenerative cells are either absent or occur in very small numbers. Musser (1914) and Archibald (1919) state that the colour index is low, but Sheard (1924) cites cases in which it exceeds unity, a finding which appears to be especially characteristic of the T N T group.

Clinical and laboratory examinations generally show an entire absence of evidences of hæmolysis in this disease and this is undoubtedly its most characteristic distinction from pernicious anæmia. Thus the skin is pale grey, the sclera are not icteric and the urine is not high coloured. Schneider (1918) showed that the duodenal pigment values were subnormal, urobilinogen being absent, the urobilin being less than normal and the bilirubin reduced proportionately to the anæmia. The Van den Bergh reaction in Hayes-Smith's case was always normal.

At autopsy hæmorrhages into the serous and mucous membranes may be observed and soft, light yellow, translucent, fatty marrow is found to replace the red marrow in the long, short and flat bones. Sheard (1924) has recently pointed out that red marrow often persists in certain situations so that the condition is strictly one of hypoplasia rather than of absolute aplasia of the marrow elements. Furthermore, as Tidy (1922) and others have shown, transitional forms also occur in pernicious anæmia and in his case a sharp line of demarcation was found in the femur, one side being filled with hyperplastic and the other with aplastic marrow. The same curious condition was demonstrated in the ulnar and other bones.

Clinically, too, mixed cases are met with, and, whilst extreme aplastic anæmia may be readily distinguished from classical pernicious anæmia by its clinical course, the absence of evidences of hæmolysis and of regenerative cells in the

and anæmia were extreme. Biochemical analysis yielded a total blood calcium of 9.8 mg and an ionic calcium of 8.2 mg per cent.

Hæmatological examination showed that the red blood corpuscles were 1,937,500 per cmm, the leucocytes 5,000 per cmm, the hæmoglobin 50 per cent and the colour index 1.3. The Price-Jones curve presented a broad base with marked displacement to the right, while the average diameter of the corpuscles equalled  $8.63 \mu$ . Blood smears indicated a megalocytic anæmia associated with a few microcytes and poikilocytes. No nucleated reds and but little polychromasia were observed. An indirect Van den Bergh of 0.55 unit was recorded.

At autopsy 57 days later the shaft of the tibia was decidedly thinned while its bone-marrow presented advanced aplasia, only one small patch of red marrow being present (Coloured Plate LIX, No. 7).

In the yellow marrow, no marrow cells or red blood corpuscles were seen, the microscopical picture consisting of large acellular spaces with surrounding fat cells. Smears of the red marrow, however, still indicated foci of activity. Normoblasts were fairly numerous, many of the red cells being of normal size and shape, but megalocytes were not common in the specimens studied. Myelocytes with fine eosinophilic granules were commonly observed.

S 88 R W, an Anglo-Indian, male, aged 59 years was admitted to St George's Hospital on the 13th June, 1927. He was a typical case of chronic sprue of some 3 to 4 years' duration and during this period he had lost 4 stone in weight.

Biochemical examination showed a total and ionic blood calcium of 10.1 and 8.6 mg per cent respectively.

On the 17th June, 1927, the blood corpuscles were 3,412,500 per cmm, the leucocytes 7,500 per cmm, the hæmoglobin 75 per cent and the colour index 1.1. The blood picture showed some anisocytosis, but no other abnormality. The indirect Van den Bergh reaction was only 0.4 unit.

Under treatment the patient improved and left hospital, but he was re-admitted on the 4th August, 1927, in a moribund condition and died.

At autopsy 49 days after the first hæmatological examination was made the central canal and the cancellous tissue spaces of the tibia were found to be filled with pale yellow marrow only a few points of red marrow being observed while microscopical examination showed nothing but fat cells (Coloured Plate LVIII, No. 8).

### (C) *Commentary on the Bone-Marrow Findings*

Price-Jones (1922) suggested that three types of red cells may be met with in the peripheral blood, the normal sized corpuscles resulting from a normal stimulation of healthy marrow, the abnormally large cells arising from some abnormal excitation of the bone-marrow and the small erythrocytes produced by over stimulation of the bone-marrow owing to abnormal blood destruction or blood loss. All these corpuscles may occur in sprue but the last variety, i.e., the microcytes, are decidedly rarer than the other types—a finding which is in line with our general conclusion that deficient blood production rather than excessive blood destruction underlies the anæmia in this disease.

While in both sprue and primary aplastic anæmia stigmata of regeneration such as nucleated red cells and polychromatophilia are frequently absent from blood smears, they differ in regard to the colour index which is generally stated to be below 1 in the latter condition. What then is the explanation of this anomaly?

PROTOCOL I—*contd*

Case No	Age	Sex	Duration of disease.	Red blood corpuscles per c mm	Percentage of Hæmoglobin	Colour index	Indirect Van den Bergh reaction
S 22	58	F	3 months	2,933,333	60	1 02	
			4 months	1,933,333	55	1·42	
S 41	45	M	3 months	2,140,000	75	1 75	0·5
S 21B	45	M	3 months	1,490,000	25	0·84	1 0
S 19	29	M	Few months	5,050,000	90	0·89	0·25
S 17	57	M	4 months	3,700,000	80	1 08	
S 36	37	M	4 months	3,920,000	80	1 02	0·25
S 91	43	F	4½ months	5,125,000	95	0·93	0·28
S 30	40	M	5 months	2,616,666	60	1·15	0·7
S 50	33	F	5 months	1,600,000	40	1 25	
S 47	19	M	6 months	1,700,000	50	1 47	
S 16A	38	M	6 months	3,600,000	75	1 04	0 66
S 72	29	M	6 months	3,300,000	75	1 14	1 0
S 35	51	M	8 months	4,270,000	85	1 0	0·75
			9 months	4,433,333	80	0·9	
S 76	48	M	8 months	3,983,333	65	0·82	1 2
			3 years	4,190,000	88	1 05	0·4
			4 years	2,737,500	70	1 28	
S 88	59	M	8 months	3,412,000	75	1 1	0·4
S 2	44	M	9 months	575,000	10	0·87	
S 21A	27	M	9 months	1,462,500	35	1·2	1 0
			10 months	1,616,666	25	0·77	
			12 months	941,666	15	0 8	
S 71	38	M	9 months	2,533,333	65	1 28	0·8
S 90	23	F	9 months	1,450,000	30	1 03	0·4
S 96	34	M	9 months	4,400,000	86	0·98	
S 21	25	M	10 months	3,750,000	80	1 07	0·2
S 31	37	F	10 months	3,764,285	80	1 06	0·4
S 18	44	M	11 months	4,200,000	70	0·83	0·25



neither megaloblasts nor normoblasts appear in the peripheral blood and remissions do not occur

## IX SUMMARY AND CONCLUSIONS

1 The red blood counts, hæmoglobin estimations and colour indices in 67 cases of sprue are recorded, and their group distributions analysed. The corpuscles averaged 3,242,000 per c mm, the hæmoglobin 65.5 per cent, and the colour index 1.0 for the whole series.

2 At onset sprue anæmia is rarely if ever found to be as severe as that met with at a corresponding stage in pernicious anæmia, and during its subsequent course a grave grade of anæmia less frequently develops. Only 17.9 per cent of our series showed red cell counts of under 2,000,000, though in two exceptional instances counts of 575,000 and 400,000 per c mm were recorded.

3 Throughout all stages of the diseases 41 or 61.2 per cent of the cases showed a colour index equal to or exceeding unity, while in the remaining 26 values of from 0.8 to 0.99 were recorded in 19. In no instance was it less than 0.7.

4 The blood-picture proved remarkably constant. Anisocytosis was the one outstanding feature especially as regards increase in size. Microcytes were much less in evidence than the larger forms. Poikilocytosis and polychromasia occur but to nothing like the degree observed in pernicious anæmia. Nucleated red cells were rarely seen.

5 In uncomplicated sprue the leucocytic counts were either normal (27.7 per cent) or there was a leucopænia (55 per cent) which was sometimes associated with a relative lymphocytosis. Leucocytosis—indicative of some intercurrent infection or complication—was observed in 5 of the 60 cases investigated.

6 A blood crisis in sprue characterized by a rapid and critical fall in the hæmoglobin and red blood corpuscles is described. The condition is generally associated with severe diarrhœa and progresses to a fatal issue without remissions and without those evidences of corpuscular regeneration which constitute so typical a picture of the blast crises in pernicious anæmia.

7 Price-Jones curves were investigated in 11 cases of well established sprue and closely resembled those obtained in pernicious anæmia being characterized by marked asymmetry, broadening of the bases, considerable displacement to the right (10 cases) and definite increase in the mean diameter of the corpuscles ( $8.07 \mu$ ). Essentially the anæmia is of megalocytic type.

8 The Van den Bergh reaction was investigated in 48 cases. Of these, 54.17 per cent gave readings under 0.6 units, 72.9 per cent under 0.8 and 91.7 per cent under 1.2 units. In only 4 cases (8.3 per cent) did the results exceed the latter figure and in 3 of these there was evidence of intercurrent malaria. The mean obtained for the whole series was 0.66 unit.

9 A comparison of these results with those obtained by reliable authorities in health as well as in cases of malaria and active pernicious anæmia shows that hyperbilirubinæmia is a far more frequent finding in the two latter diseases. From a clinical viewpoint data afforded by the Van den Bergh reaction are often

## PROTOCOL I—concl'd

Case No	Age	Sex	Duration of disease	Red blood corpuscles per c mm	Percentage of Hæmoglobin	Colour index	Indirect Van den Bergh reaction
				2,500,000	50	1·0	
				3,166,666	75	1·18	
S 78	36	F	Chronic	4,660,000	90	0·97	.
S 80	32	M	Chronic	1,762,500	40	1·13	3·0
				400,000	10	1·25	2·0
Average count for the series				3,242,000	65·5	1·0	0·66

Regarding technique the red blood corpuscles and leucocytes were counted by means of the Thoma-Zeiss hæmocytometer, and the hæmoglobin percentage by Sahli's hæmoglobinometer

(a) *The Red Blood Corpuscles*

The average number of corpuscles found in the 67 cases of our series equalled 3,242,000 per c mm. The lowest initial count of 575,000 corpuscles per c mm occurred in a fatal case (S 2) of 9 months duration, and together with a terminal count of 400,000 per c mm recorded in another of our patients (S 80) constitute the two lowest estimations so far reported in sprue. The highest count, i.e., 5,500,000 per c mm was obtained within two weeks of the onset of the disease.

A group classification of the results show that —

1 case or	1·5	per cent	gave readings under 1,000,000 per c mm			
12 cases	„	17·9	„	„	„	2,000,000
31	„	41·3	„	„	„	3,000,000
49	„	73·1	„	„	„	4,000,000
61	„	91·0	„	„	„	5,000,000
6	„	9·0	„	„	„	over 5,000,000

The six cases in the last group were all examples of early sprue, two having had symptoms for less than 1 month, two for less than 2 months, and the remaining two for 4 and 4½ months respectively.

The incidence in the different groups is depicted diagrammatically in Plate LXIX. It will be noted that the greatest number of cases, i.e., 37 occur in the 2 to 4 million groupings. In pernicious anæmia, on the other hand, Cabot (1927) has shown that 84 per cent of American and 89 per cent of foreign cases have a count of under 2,000,000 corpuscles per c mm at the first examination. In only 17·9 per cent of our patients was this the case.

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Analysis shows that in 41 cases or 61·2 per cent the colour index either equalled or exceeded unity, while in 26 or 38·8 per cent it was less than 1. In the latter group, however, it was really never low for no case was less than 0·7, and in 19 out of the 26 values of from 0·8 to 0·99 were recorded.

During the first few weeks of illness the colour index was found to be under 1 in six out of seven cases, but shortly afterwards high colour indices became established, and thus was the general finding throughout the disease. Thus in 39 cases of under one year's duration, the colour index equalled or exceeded unity in 23 or 59 per cent, while in a group of 28 patients in whom the disease had been established for a period of from 1 to 10 years, eighteen or 64·3 per cent showed a similar result.

In pernicious anaemia Cabot (1927) has recorded that the colour index varies at different phases of the disease, and that in its worst stages a high colour index is present in 88 per cent of cases. On the other hand during remissions, colour indices equalling or exceeding unity were met with in only 49 per cent of his series. At no stage would the blood picture in a case of pernicious anaemia be regarded as of secondary type merely because the colour index was found to be less than 1, and in our opinion the same criteria hold in sprue.

#### (d) *The Blood Picture in Sprue*

In the early weeks of the disease blood changes are not marked and slight degrees of anisocytosis may be the only change observed in stained films.

In the more advanced cases on the other hand anisocytosis is a very definite and constant characteristic, the megalocytic types preponderating although a few microcytes may also be observed.

In a consecutive series of 28 of our cases anisocytosis especially as regards increase in size was noted in 22, some grade of poikilocytosis in 16, and polychromatophilia in five. Nucleated red cells are either entirely absent or present only in very small numbers, and in the majority of our films they have not been noted. Gulland and Goodall (1925) have pointed out that in pernicious anaemia, megaloblasts are only found with anything like ease when the red cell count is below 2,500,000 per cmm. This fact might explain their absence in many of our less anæmic cases, but there still remains a group with blood counts below this figure in which deficient production must be the underlying factor. Many observers have noted the relative infrequency of nucleated cells in sprue when compared with pernicious anaemia, but in 8 of their 12 recent cases Newham, Morris and Manson-Bahr (1926) report having found from 1 to 3 normoblasts or megaloblasts to every 500 leucocytes counted.

In epitomising our findings we would describe the blood picture in sprue as that of a megalocytic anaemia with minimal signs of regeneration, a result which is in conformity with the aplastic condition of the bone-marrow so frequently met with at autopsy. In the exceptional cases where nucleated red cells are demonstrable, the red marrow is probably in a hyperplastic condition.

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## EXPLANATION OF PLATE LXVIII

- Figs 1 and 2 Blood picture of sprue cases showing megalocytic changes
- Fig 3 From an advanced case of pernicious anæmia
- „ 4 The same case showing reticulocytes

[The Coloured Plates representing the bone-marrow appearances referred to in the text may be found with the foregoing paper, in this number, entitled "The Morbid Anatomy of Sprue" (pp 799—825) (*See Coloured plates LVIII and LIX*)]

PROTOCOL II—*contd*

Case No	Total leucocytes per cmm	DIFFERENTIAL COUNT PERCENTUM				
		Polymorpho-nuclear leuco-cytes	Eosinophile leucocytes	Lympho-cytes	Large mono-nuclears	Transitional mononu-clears
S 29	4,062	40.5	0.2	49.3	9.8	0.2
S 30	7,812	55.6	1.5	34.9	6.9	1.1
S 32	9,375	64.4	1.1	24.8	9.7	0.0
S 33	10,000	49.8	1.4	38.4	10.4	0.0
S 34	5,312	47.8	0.8	49.0	1.8	0.6
S 37	6,875	37.5	11.9	37.5	11.3	1.8
S 38	11,562	59.7	1.6	33.3	5.4	0.0
S 42	13,750	81.7	0.5	14.2	2.1	1.4
S 50	4,375	22.2	1.0	69.5	4.0	3.3
S 16A	10,312	45.6	4.2	45.6	2.8	1.8
S 21A	5,937	54.1	1.7	42.5	0.4	1.3
S 21B	5,937	81.4	0.2	17.0	1.2	0.2
S 33A	4,375	70.9	1.8	22.2	3.1	2.0
S 70	10,900	30.4	0.8	59.5	2.4	6.9
S 71	20,312	78.5	0.5	18.5	2.0	0.5
S 72	4,062	51.8	0.9	38.6	5.7	3.0
S 76	5,937	48.4	1.0	47.6	1.4	1.6
S 77	7,500	63.6	0.2	31.2	2.8	2.2
		42.6	0.2	54.2	2.0	1.0
S 78	8,437	38.0	2.0	57.0	1.0	2.0
S 79	3,750	52.4	2.2	42.4	2.0	1.0
S 80	4,375	55.2	0.6	42.8	0.8	0.6
	2,812					
S 81	5,625	59.3	1.1	36.0	1.6	2.0
S 82	4,357	57.0	1.0	40.0	2.0	
S 83	17,500	81.3	1.0	16.3	0.4	1.0
S 84	6,250	79.8	3.2	15.2	0.4	1.4
S 85	3,125	82.4	0.6	15.0	1.8	0.2



there is an absolute and relative increase in the neutrophile granular leucocytes which is certainly not characteristic of uncomplicated sprue, but indicates some intercurrent infection. This may be either obvious or occult and, in the latter case, careful clinical investigation is required to reveal it.

As a general rule marked leucopænia occurs only in the patients showing most anæmia, and of the 11 cases in which the total leucocytes numbered less than 4,000 per c mm, 10 showed a red blood count of under 3,000,000 erythrocytes.

### PROTOCOL III

#### *The leucocytic groups in 60 cases of sprue*

Number of Group	Number of Leucocytes per c mm	Number of cases	DIFFERENTIAL COUNT PER CENT				
			Neutrophile Polymorphonuclear Leucocytes	Eosinophile Leucocytes	Lymphocytes	Large Mononuclears	Transitional Mononuclears
Group 1	1,000—5,900	33	53.2	1.7	40.2	3.3	1.5
Group 2	6,000—11,900	22	54.8	2.0	37.2	3.8	2.1
Group 3	12,000—20,900	5	77.0	1.2	19.0	1.7	1.0

### V BLOOD CRISES IN SPRUE

A crisis may be defined as the turning point in a disease indicating recovery or death, and, used in this sense, blood crises in sprue are not infrequently met with in the tropics. In certain of our cases, we have observed a rapid and critical fall in the hæmoglobin and red blood corpuscles associated with severe diarrhoea and progressing to a fatal issue without any evidence in the peripheral blood, either of hæmolysis or of regenerative efforts on the part of the bone-marrow. In this respect, the blood crisis in sprue is the reverse of the blast crisis in pernicious anæmia where megaloblasts and normoblasts are usually in evidence and polychromatophilia and anisocytosis constitute marked characteristics of the blood smears. Furthermore, in sprue a blood crisis is invariably a terminal event, whereas it not infrequently heralds a definite remission in pernicious anæmia.

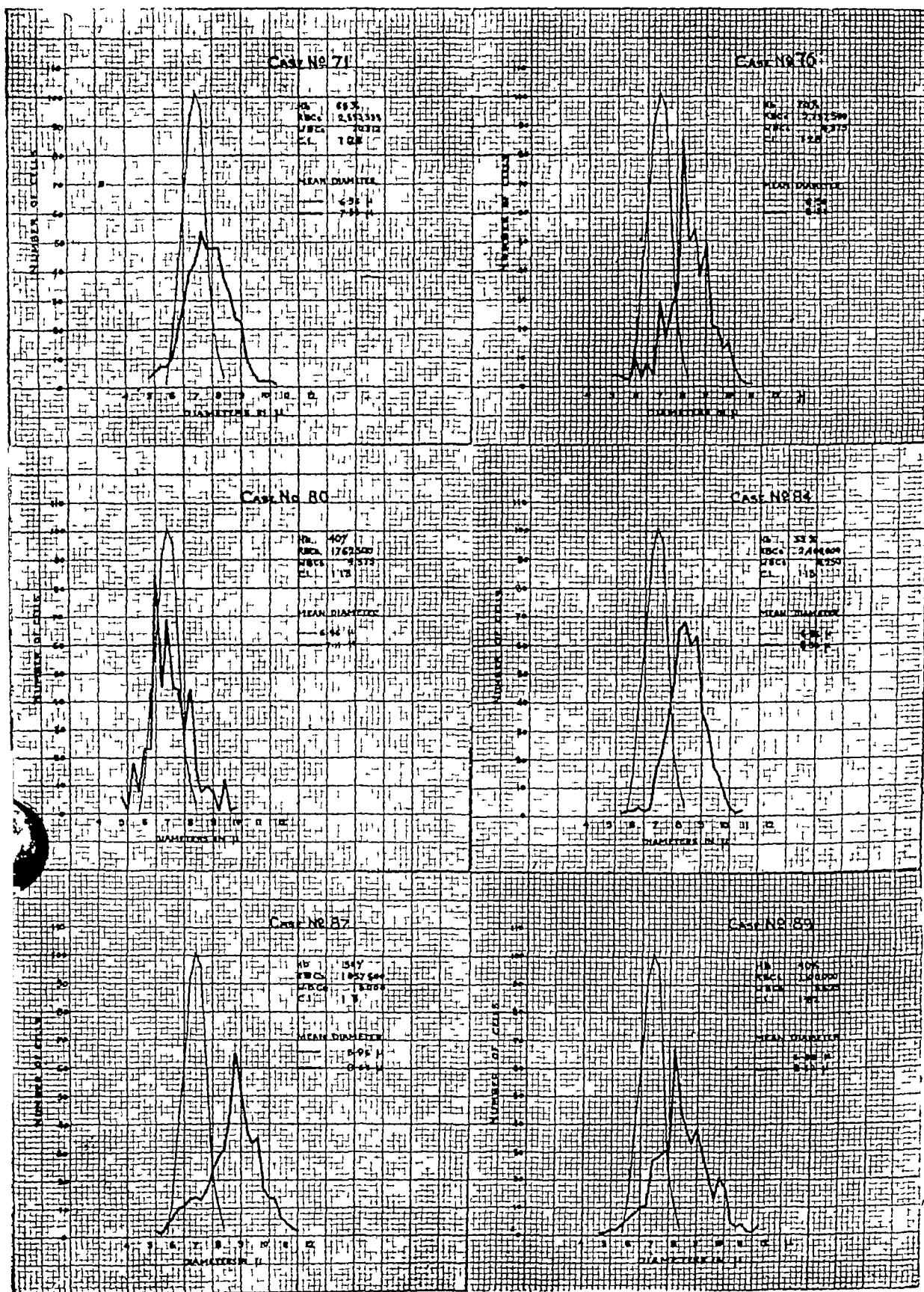
We have met with several striking examples in Bombay, and the following case history may be cited as illustrating the clinical findings, though in some patients the downward gradient of the anæmia is even more rapidly established.

S 27 Mrs A, an Anglo-Indian, aged 35 years, was first admitted to St. George's Hospital, Bombay, on the 13th August, 1923, complaining of an ulcerated tongue, the frequent passage



# PLATE LXX

Price-Jones' Curves of the Blood of Sprue Cases



in groups progressing by measurements of  $0.25 \mu$ . These results were plotted graphically, the abscissæ being graduated in terms of the diameters of the corpuscles and the ordinates according to the number of cells measured. The mean diameter of 500 cells was taken to represent the mean diameter of the red cells of the specimen, and in 1922 Price-Jones showed that the average of these measurements in 20 cases of pernicious anæmia ( $8.24 \mu$ ) exceeded that observed in normal individuals ( $7.2 \mu$ ).

In health, blood cells showed minimal variations ( $6.0$  to  $8.75 \mu$ ) and a characteristic curve was obtained with a defined peak and narrow base, whereas in pernicious anæmia the whole curve moved to the right and presented a wide base owing to the marked variations in the size of the corpuscles ( $4$  to  $12 \mu$ ). There was a shift to the left in secondary anæmia depending on the lower mean diameter of the red cells ( $6.85 \mu$ ) while the base was definitely broadened, the range varying from  $5.0 \mu$  to  $8.5 \mu$ .

Hampson and Shackle (1924) described the Price-Jones curves in three cases of sprue and found marked displacement to the right. The mean diameters of the corpuscles were  $8.39 \mu$ ,  $8.87 \mu$  and  $9.0 \mu$  and in their cases a typical megalocytic condition of the blood was observed. Newham, Morris and Manson-Bahr (1926) examined 10 cases of sprue, but in only three did the curves approximate to those described for pernicious anæmia, and even here the marked irregularity and widely extended base so characteristic a feature of pernicious anæmia were not present. The curves tended to return to a more normal type as the anæmia decreased.

### *Analysis of results*

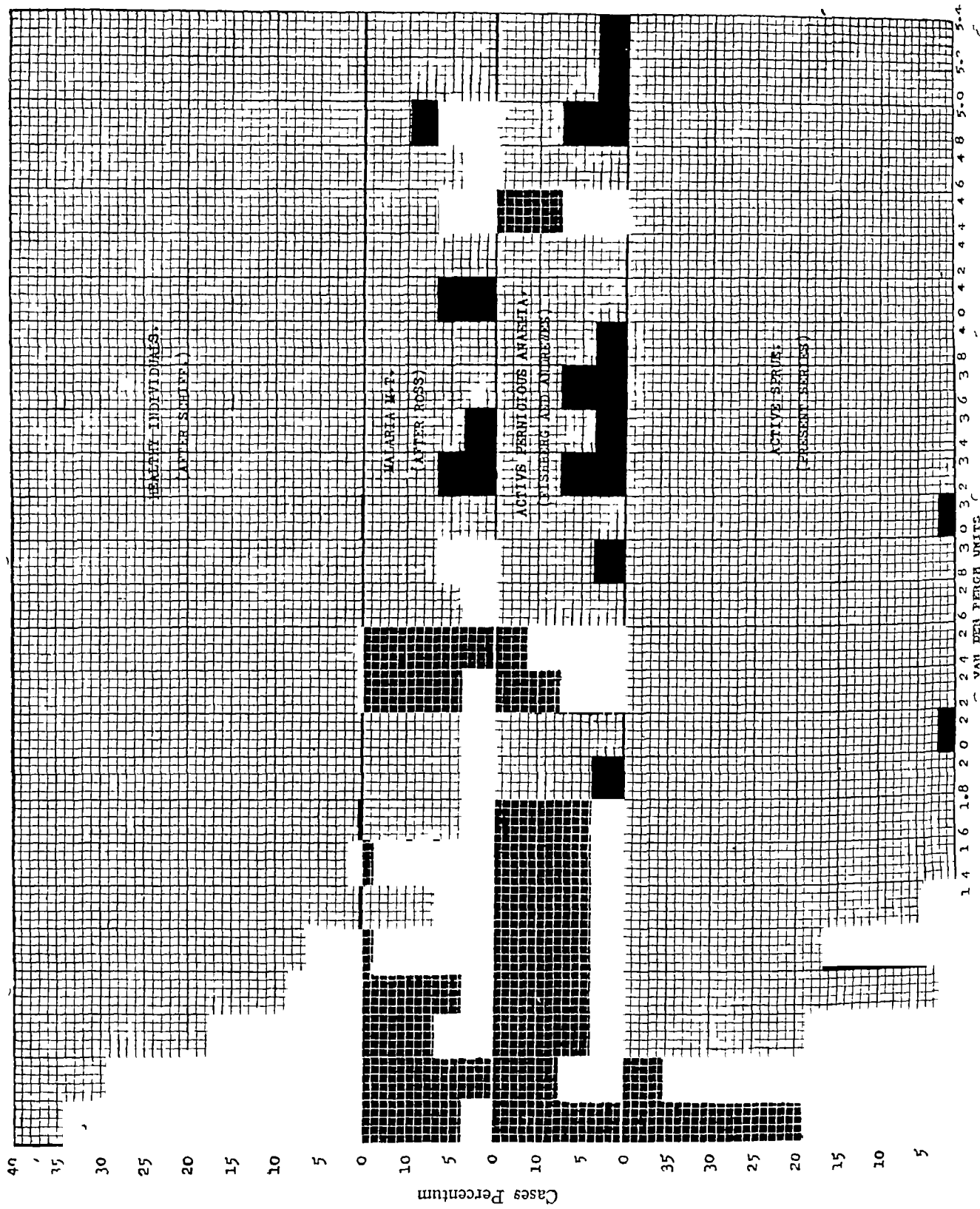
In our series, the Price-Jones curves were investigated in 11 instances and the graphs may be studied in detail in the accompanying Plates LXX and LXXI.

All the patients were suffering from a well established anæmia, the hæmoglobin varying from 30 to 70 per cent and the red blood corpuscles from 1,100,000 to 3,075,000 per c mm. In 10 out of 11 instances, the colour index exceeded unity.

The average of the mean diameter of the red blood corpuscles for the whole series was considerably above normal and equalled  $8.07 \mu$ , one result was only  $7.11 \mu$ , but in the remaining 10 cases the lowest equalled  $7.55 \mu$  and the highest  $8.68 \mu$ .

In 10 out of 11 instances the curve was definitely displaced to the right (see Plates LXX and LXXI), while in the exception (No. 80) though its base was broadened the apex lay to the left of the normal. The mean diameter of the corpuscles fell within average limits (i.e.,  $7.11 \mu$ ). This case is of exceptional interest as the patient returned to hospital some four months later with the most profound anæmia yet recorded in sprue, the blood corpuscles being 400,000 per c mm, the hæmoglobin 10 per cent, and the colour index 1.2. At autopsy the tibia was filled with hyperplastic red marrow similar to that observed in pernicious anæmia. On the other hand in another fatal case (No. 87) which showed

# A Comparison of Reported Van den Bergh Readings in Malaria Sprue, Pernicious Anemia, and Healthy Individuals



*The nature and mechanism of the reaction*

The test is really a modification of the Ehrlich-Proscher reaction, and is due to the formation of azobilirubin from bilirubin when the latter substance reacts with diazotized sulphanilic acid. If a bluish-violet reaction develops within 30 seconds on adding diazo reagent to the serum, an immediate direct reaction is registered, but if the appearance of a reddish colour gradually deepening into violet is delayed for 1 to 15 minutes or longer, delayed direct reaction is recorded. Human bile gives the direct reaction but not human serum. When human serum is treated with alcohol, however, a reaction with diazo reagent occurs characterized by the appearance of a reddish violet colour reaching its maximum intensity almost at once. This constitutes a positive indirect reaction. By colorimetric measurement and comparison against a given standard the test has been placed on a quantitative basis, the results being expressed in units, 0.5 mg per 100 ccs constituting one Van den Bergh unit (1/200,000).

Thannhauser and Anderson (1921) have improved the quantitative estimation of bilirubin in the indirect reaction by first mixing the diazo reagent with the test plasma and then subsequently adding 95 per cent alcohol and saturated ammonium sulphate. By this means all azobilirubin is retained in the supernatant fluid, loss in the albuminous precipitate being avoided.

According to modern opinion hæmoglobin liberated from disintegrated red blood corpuscles is not excreted in the urine unless the renal threshold for this blood pigment be exceeded. This is only occasioned by an exceptionally severe and rapid intra-vascular hæmolysis such as occurs in black-water fever or paroxysmal hæmoglobinuria. Under normal and most pathological conditions occurring in the body, defunct corpuscles are phagocytosed by the cells of the reticulo-endothelial system—especially those of the spleen, bone-marrow and liver—and converted into bilirubin which circulates in the blood firmly bound to the serum proteins possibly by adsorption. This is the combination of bilirubin which reacts with diazo reagent to give the indirect Van den Bergh test, and which is so definitely increased in the hæmolytic anæmias. Further in this form bilirubin is not excreted by the kidney, but on reaching the liver it is extracted by the hepatic cells, split from its protein combination and excreted as simple bilirubin in the bile. According to Rosenthal (1925) it is the bile salts which produce separation of the bilirubin from its adsorption compound in the liver.

*The Bilirubin Content of Normal Sera*

The earlier workers including Van den Bergh (1918), Lepehne (1920), McNee (1923) and Andrewes (1924) regarded the normal variations in the bilirubin of the sera derived from healthy people as being from 0.2 to 0.6 units, i.e., 0.1 to 0.3 mg per 100 ccs, while Fishberg (1926) stated that normal bilirubin is less than 0.8 units, most often well under 0.5 units. Others, however, have recorded higher upper normal limits, Cowen (1925) placing it at 0.5 mg per 100 ccs (1 unit) and Weimer (1926) regarding the figures at 0.75 mg per

justification for regarding *Monilia albicans* as the cause of the disease owing to its constant presence in the tongue, saliva, intestinal mucus and stools of sprue patients and its rarity in the faeces of normal individuals and of those suffering from other forms of diarrhoea. Furthermore, their presence afforded a reasonable basis of explanation for the acid gaseous stools characteristic of this disease. Later this writer (1923) failed to culture the monilia from early cases as well as from autopsies on sprue patients dying in England, and in consequence now holds and has for some years held the view that the moniliasis is at most only a secondary or terminal infection. Despite these facts Michel (1927) and others erroneously continue to quote this well-known authority as supporting the fungoid theory of sprue!

Ashford in 1915 isolated a yeast (*Monilia psilosis*) from the inflamed tongue of a case of sprue and also described its cultural reactions and pathogenicity for rabbits. Later observations were extended to other laboratory animals. Subsequently he isolated this monilia from the faeces of a large series of sprue cases, and advocated the view that this disease was essentially a moniliasis of the digestive tract. Later still Ashford (1922) expressed the opinion that the mycosis was engrafted upon a state of deficiency in certain essential food elements. The latter condition resulted in a decreased output of digestive fluids and a high acidity of the intestinal contents—a state of affairs which enabled this monilia to produce the classical picture of sprue. In a series of 573 typical cases, 501 or 87.4 per cent were reported as showing laboratory evidence of infection with *Monilia psilosis*.

Martinez (1916) prepared an antigen from three strains of monilia isolated from Ashford's cases, and utilizing a carbol saline suspension of a three days' culture grown on Sabouraud's glucose agar reported favourable results with the complement fixation test. Four cases of sprue gave positive reactions, while 12 sera sent in for the Wassermann test served as controls and were all negative. Michel (1917) insisted that old cultures made the most potent and stable antigens, and advocated watery extracts of colonies grown for four weeks on Sabouraud's media. In his large series every case which was diagnosed clinically as sprue and from which *Monilia psilosis* was isolated gave a positive complement fixation test. In chronic and latent sprue, however, and in cases which had recovered the test tended to become negative. Both the Noguchi and the ordinary Wassermann technique were employed, the former being regarded as the more delicate. Both Martinez (1920) and Michel (1927) have reiterated their advocacy of this test not only as a laboratory aid to diagnosis, but as an index to the progress of cases under treatment. Michel (1927) recently recorded that in a series of 100 patients who were diagnosed as sprue by Ashford on clinical and mycological grounds, the serological reactions were positive in ninety-eight (98 per cent).

The findings of the Porto Rico school have received some measure of support from Dold (1917), Smith (1924) and others who have isolated monilia from sprue faeces in a variable proportion of cases, but serious criticisms regarding

*Commentary*

All our cases were bed patients ill in hospital with sprue. They were not being investigated during a remission period, but either in the first attack or during relapses, and considering the incidence of malaria in Group 4 and the varying severity of the anæmia represented in this series, we must regard the presence of a definite pathological bilirubinæmia as a relatively uncommon feature of uncomplicated sprue.

When these results are compared with those obtained during corresponding periods in pernicious anæmia, the low values of the indirect Van den Bergh readings in our series are still more striking. During the remission periods in pernicious anæmia, i.e., the stages of rapid temporary improvement, all will agree that the bilirubin content of the serum falls considerably and sometimes reaches even a normal level, but this is certainly exceptional during the active phases of this disease when readings of from 2.0 to 5.0 units are commonly observed. The published figures of Van den Bergh (1919), McNee (1923), Andrewes (1924), Cowen (1925), Fishberg (1926) and others show this to be the case. During active relapse high indirect Van den Bergh readings are the rule rather than the exception in pernicious anæmia, whereas in sprue a reverse state of affairs is found to exist, and in our opinion the clinician would be ill-advised in doubtful cases were he to neglect taking cognisance of the important data afforded by this test—especially where low bilirubin values are obtained.

At the same time we would direct attention to our post-mortem findings where hyperplasia of the red marrow associated with a limited deposit of iron containing pigment in the viscera was occasionally demonstrated. Here, no doubt, an increase in the bilirubin content of the serum did occur. The hæmosiderosis, however, was never as extensive as that observed in pernicious anæmia, and in view of our general findings in the Van den Bergh reaction and the frequency of aplasia in the bone-marrow at autopsy, we feel that blood destruction rarely if ever reaches a degree comparable to that observed in the latter disease and that the anæmia of sprue must be generally classified as of non-hæmolytic type.

#### VIII THE BONE-MARROW AND THE HÆMATOLOGICAL FINDINGS

In separate communication in this number of the *Journal* dealing with the morbid anatomy of sprue (pp 799—825), various pathological changes including hyperplasia and aplasia of the bone-marrow are described in detail and coloured drawings, (Coloured Plates LVIII and LIX) indicating their different naked eye appearances are appended. It will be necessary on more than one occasion to refer the reader to these coloured plates when dealing in the present section with the hæmatological features of the peripheral blood and the bone-marrow findings at autopsy.

##### (A) *Hyperplasia of the Bone-Marrow*

Hyperplasia of the bone-marrow was met with on three occasions. Once it was of moderate grade. In the two other instances it was very advanced, as is illustrated in the following case histories,—

similar range of dilutions for hæmolytic action, but even in the most concentrated solutions this was never shown

### Technique A

Two different methods were employed. In Technique A various sera derived from normal, syphilitic and sprue cases were tested against a series of ascending dilutions of antigen in the presence of a constant quantity of complement, 12 tubes being employed for each serum. An additional control tube to detect any anti-complementary tendency in the serum itself was included. Each tube contained one volume of a varying dilution of antigen (1/1 to 1/200), one volume of complement (3 M H D's), one volume of saline (85 per cent) and one volume of heated de-complemented serum (1/5 dilution). This system was incubated for 1 hour at 37°C and then one volume of sensitized red blood corpuscles was added. Readings were made when the complement, antigen, serum and negative serum controls had completely hæmolyzed. Known positive sera derived from rabbits inoculated with monilia cultures were also included, but with this species of animal it was found necessary to use a somewhat larger quantity of complement per unit volume (4 M H D's) in order to avoid pseudo-positive reactions.

### Technique B

In the second method the above procedure was reversed, the quantity of antigen remaining unaltered, while the complement varied in an ascending series of concentration of from 2 to 24 M H D's. The actual amount of antigen utilized was about 1/3 the anti-complementary dose and generally equalled a 1/15 dilution of the original monilia extract. Unit volumes of antigen (1/15 dilution), saline, de-complemented serum and complement of increasing strength were used. A series of 12 tubes were put up for each serum and positive and negative sera, and similar controls to those employed in Technique A were included. The system was incubated for 1 hour at 37°C and then sensitized red blood corpuscles were added. Readings were again made when all the controls, including the normal sera, had hæmolyzed.

### THE SEROLOGICAL RESULTS WITH HUMAN SERA

All the sprue sera were obtained from typical cases during the active phases of the disease. Most of the patients were in hospital with relapses, but in a few the blood was collected during first attack. *Monilia psilosis* (Ashfordi) was isolated from the fæces of the earlier cases only, as Mackie's survey had shown that about 50 per cent of all the hospital population in Bombay harboured this yeast in the fæces.

### Technique A

The antigens used with Technique A were three in number. All were derived from different strains of *Monilia psilosis* (Ashfordi) isolated from cases of sprue in Bombay.

Polychromasia and platelets were scarce and no nucleated red cells were observed. The hæmatological findings indicated a profound anæmia with minimal signs of regeneration of the bone-marrow yet at autopsy the red marrow of the ribs suggested little that could be regarded as abnormal, while the medullary canal and the cancellous tissue spaces of the tibia were filled with soft hyperplastic red marrow resembling in appearance that originally described by Cohnheim as characteristic of pernicious anæmia (Coloured Plate LIX, No 6).

Smears of the bone-marrow showed numbers of nucleated red cells, but mature red blood corpuscles were not numerous. Granular cells including myelocytes and polymorphonuclear leucocytes were plentiful. Some of the cells contained phagocytosed melanin-like pigment (probably malarial).

The spleen was firm, dark and pigmented, and weighed 6½ ounces. Taken in conjunction with the various pigment deposits observed in the viscera, these findings indicate chronic malaria.

Hyperplasia of the red marrow was the outstanding feature in the gross pathology of this case at autopsy and its chief interest lies in the absence of nucleated red cells and other stigmata in the peripheral blood indicative of regenerative efforts on the part of the bone-marrow. The failure can hardly be regarded as a terminal event for it was not more in evidence 128 days earlier when the anæmia was less advanced. Furthermore, this was one of the few cases of sprue in which Van den Bergh reaction indicated that excessive hæmolysis was occurring.

Undoubtedly the blood forming organs had here to bear the brunt of two intercurrent disease—malaria and sprue—and this may explain the anomalous findings in the bone-marrow as well as the exceptionally high indirect Van den Bergh readings of 3.0 and 2.0 units. Pathological bilirubinæmia of this grade is rare in uncomplicated sprue, and it appears probable that corpuscular hæmolysis as well as the pathological condition of the bone-marrow were factors in the production of this profound anæmia. Blood transfusion, which has been advocated by Low and Cooke (1927), was performed, but admission to hospital had been too long delayed for this generally effective therapeutic procedure to be of value.

### *(B) Aplasia of the Bone-Marrow*

Aplasia or hypoplasia of the bone-marrow which was met with in 5 out of 8 cases constitutes a common and characteristic feature of the sprue autopsy. Even though normally in the adult, the red marrow is confined exclusively to the ends of the long bones, the persistence of such a distribution under the stimulus of a severe anæmia no less than its entire replacement by fat indicates a lack of response and regenerative effort on the part of the erythropoietic tissues that is remarkable. As the pathological features of aplasia have been fully described and figured elsewhere, only the epitomised histories of the hæmatological findings and the bone-marrow condition in illustrative cases are recorded below.

S 87 J F, a European woman, aged 40 years, was admitted to St George's Hospital on the 9th May, 1927, and died on the 16th July, 1927. There was a history of sprue extending over the past 2 years and associated with a loss of 7 stone in weight. The patient presented the classical lingual and gastro-intestinal features of sprue while emaciation



of sprue reacted with monilia extracts, and that, owing to the presence of associated zonal effects, they were regarded as being dependent on a non-specific adsorption of complement rather than on a specific antigen-antibody reaction. Sixteen of the eighteen non-sprue sera failed to react, but in two syphilitics partial positive reactions were registered.

# THE SEROLOGICAL RESULTS WITH RABBITS INOCULATED WITH *Monilia psilosis* (Ashfordi)

In order to test the antigenic potency of various monilia extracts, rabbits were inoculated by repeated intravenous injections of 1 c.c. of a saline emulsion of dead *Monilia psilosis* (Ashfordi) every fourth day. Specimens of blood were generally collected from the 6th to the 9th day after the sixth injection, and

## PROTOCOL II

### Complement Fixation Reactions in rabbits given repeated intravenous injections of *Monilia psilosis* (Ashfordi)

Number of rabbit	Days since inoculation started	M H D's OF COMPLEMENT								
		2	4	6	8	10	12	15	18	24
338	27	+	+	±	0	0	0	0	0	0
354	29	+	+	±	0	0	0	0	0	0
361	25	+	+	+	+	+	+	+	+	±
362	25	+	+	+	+	+	+	+	+	+
371	13	+	+	+	+	+	+	+	+	+
374	26	+	+	+	+	+	+	+	0	0
376	26	+	+	+	+	+	+	+	+	+
377	10	+	+	+	+	+	+	+	+	+
378	32	+	+	+	+	+	+	+	+	+
379	27	+	+	+	+	+	+	+	+	+
380	28	+	+	+	+	+	+	+	+	+
381	28	+	+	+	+	+	+	+	+	+
383	30	+	+	+	+	+	+	+	+	+
384	30	+	+	+	+	+	+	+	+	+
Control		NR	0	0	0	0	0	0	0	0
Control		NR	0	0	0	0	0	0	0	0
Control		NR	0	0	0	0	0	0	0	0

+ = no hæmolysis, ± = partial hæmolysis, 0 = complete hæmolysis

At autopsy we have noted the variable condition in the bone-marrow which may be moderately to extremely hyperplastic in some instances, hypoplastic and aplastic in others

Furthermore, the aplastic yellow marrow is frequently jellified, sometimes possess a greenish tint, and occasionally is associated with definite thinning of the shaft of the long bones. Such changes suggest that the condition of the marrow met with at autopsy may not be representative of its earlier pathological state. We incline to the view that the primary response of the red marrow to sprue toxin is a hyperplasia of pernicious, i.e., megalocytic type and that only later, as the erythroblastic response fails, does it present the appearances characteristic of aplasia. Under such circumstances it is not surprising that in the later stages remnants of this erythroblastic tissue persist in the ribs, vertebræ, sternum or elsewhere and continue to manufacture a relatively high proportion of megalocytes and larger sized cells. Further investigation of the whole osseous system is necessary along these lines, but in the meantime such a view affords a reasonable basis of explanation not only of the various macroscopical lesions of the bone-marrow as transitional forms, but also of the high colour indices and the Price-Jones curves resembling those observed in pernicious anæmia and met with in sprue patients whose bone-marrow is found to be hypoplastic or aplastic at autopsy.

Briefly stated, we recognize three stages in the evolution of the bone-marrow lesions and the blood picture in sprue —

1 At the onset and during the first few weeks of the disease the bone-marrow changes are minimal, while the blood shows only slight grades of anæmia associated with a colour index varying from 0.7 to 1.0. The bilirubin values fall within the normal limits.

2 An intermediate stage of hyperplasia of the red marrow which in extreme cases may actually resemble that observed in Addisonian anæmia and frequently terminates in aplasia. There is now a considerable reduction in both the hæmoglobin and the number of red blood corpuscles, the latter generally being the more affected. In type the anæmia is definitely megalocytic, the Price-Jones curves are similar to those recorded in pernicious anæmia, and the colour index generally exceeds unity. Blood smears may show some evidence of regenerative effort on the part of the bone-marrow and increased hæmolysis may also occur, but both these features are certainly less marked than in cases of pernicious anæmia presenting similar bone-marrow lesions (*vide* Case S. 80).

3 A well established and late phase of hypoplasia or actual aplasia of the red marrow characterized clinically by a severe anæmia which remains megalocytic in type. Blood smears show an absence of nucleated red cells and other evidences of marrow regeneration, and low bilirubin readings are obtained. The colour index generally exceeds 1, and in this respect resembles the aplastic anæmia produced by T. N. T. poisoning. It is in this stage that the blood crises of sprue are generally observed, the anæmia rapidly progressing like true aplastic anæmia to a fatal termination. Unlike the blast crises in pernicious anæmia,

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of considerable value in differentiating sprue and pernicious anæmia, while the results also indicate that the anæmia must be classified as of non-hæmolytic type

10 Three stages in the evolution of bone-marrow lesions are recognized, the first being at onset when the marrow changes are minimal and only slight anæmia is present

11 An intermediate stage occurs characterized by an erythroblastic hyperplasia of the red marrow of variable intensity but sometimes culminating in a condition akin to that observed in pernicious anæmia. There is considerable reduction in hæmoglobin and corpuscles, the colour index is generally high and the anæmia is definitely of megalocytic type. The blood may show stigmata of regeneration and hæmolysis, but both features are decidedly less marked than in pernicious anæmia

12 A late phase of hypoplasia or actual aplasia of the marrow is the one most commonly observed at autopsy. It is characterized by a severe megalocytic anæmia, a high colour index, an absence of evidences of corpuscular regeneration and low Van den Bergh readings

13 It is suggested that the large sized corpuscles demonstrated by the Price-Jones curves during this last phase have their origin in small islets of erythroblastic tissue scattered through an atrophic bone-marrow

14 Deficient blood production rather than excessive blood loss constitutes the basis of sprue anæmia. In our opinion the trouble starts in an ill nourished bone-marrow which, poisoned by sprue toxin of alimentary origin, undergoes a primary hypertrophy and secondary atrophy. In the production of the latter state toxins produced by a changed intestinal flora and secondary bacterial invaders may play a part. Change in the circulating corpuscles are a minor consideration—at least in so far as they concern the mechanism of this most fatal complication of sprue—aplastic anæmia

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# A COMPARATIVE HISTOLOGICAL STUDY OF THE SPLEEN OF VARIOUS VERTEBRATES WITH REFERENCE TO THE BONE MARROW AND THE BLOOD

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IN an attempt to elucidate the function of the spleen, it was thought that some knowledge of the part played by the different portions of the spleen might be gained from a histological examination of the spleens of vertebrates beginning with the lowest forms available. In order to determine the significance of the Malpighian tissue and the pulp of the spleen, it would be necessary to examine these phylogenetically in relation to the development of the cellular elements both in the bone marrow and in the blood. The present report is a result of work done along these lines with the limited material at our disposal.

## METHODS

### 1 Stains used —

- (1) Leishman, (2) Giemsa, (3) Wright's blood stain, (4) Hæmatein and eosin, (5) Heidenhain's iron hæmatoxylin and acid fuchsin, (6) Van Gieson and hæmatein, and (7) Unna's modified orcein method

### 2 Examination of cellular elements by film preparations

### 3 Cell counts by hæmacytometers

### 4 Examination of cellular elements by marrow films

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*Reptiles**Tortoise*

*Blood*—Lymphocytes occur in large excess and have very nearly the same percentage as those in frog's blood. Polymorphs in the tortoise are greatly reduced. Transitional leucocytes are scarce. Eosinophils are abundant and are as high as 28 per cent.

*Bone marrow*—Transitional myelocytes in the bone marrow of the tortoise are peculiarly scarce. Oxyphil myelocytes are very high in number and are in a far greater percentage than the oxyphil leucocytes in the blood. The tortoise marrow is more developed and seems to have a greater quantity of hæmopoietic elements than the marrow of frog.

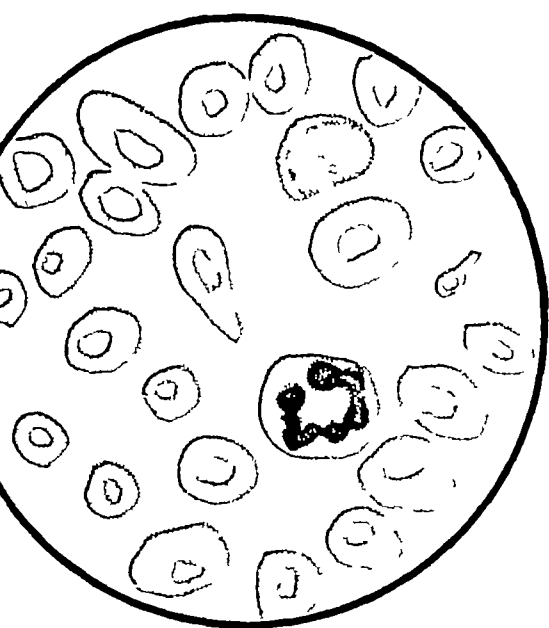
*Spleen smear*—Spleen smear of the tortoise contains about 78 per cent of lymphocytes. Polymorphs and transitionals are practically absent. Eosinophils in spleen occur to a less extent than those in the blood. The spleen of the tortoise appears less erythropoietic than that of the frog.

*Spleen section*—The spleen of the tortoise presents a pulpy appearance though to a less extent than that of the frog. The spleen has a very thin fibrous capsule, projections of which give off poorly developed trabeculae which are fibro-muscular in nature. In sections of this spleen rudiments of Malpighian follicles are present. They are scattered throughout the section and are not markedly differentiated from the pulp tissue. In this spleen a few blood vessels with hypertrophied endothelium are seen. Numerous lymphocytes are seen surrounding the vascular structures and it would appear that these structures in their fullest development represent the rudimentary Malpighian follicles in this species.

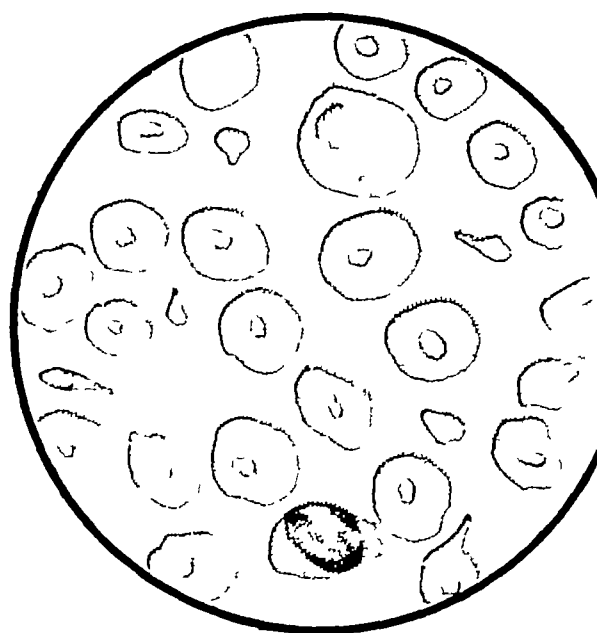
*Aves**Hen*

*Blood*—Lymphocytes in the blood of the hen are as abundant as in the frog and tortoise. Eosinophils are still more abundant in this species than in the two previously described, the percentage being nearly 38. It is worthy of note that polymorphs are greatly reduced in number forming only 0.5 to 1 per cent of the total white blood cells. In this animal as well as in the tortoise, eosinophils seem to have taken the place of polymorphs in number.

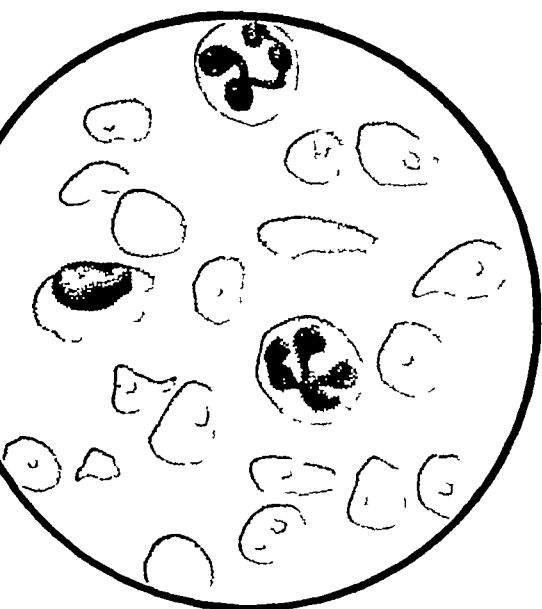
*Bone marrow*—Bone marrow in this species is more developed than in the case of the frog and tortoise. Polymorphs and transitional myelocytes are extremely rare. Oxyphil myelocytes are very numerous, their percentage being about 42, a figure which is higher than that which obtains for eosinophils in the blood of this species. The lymphocytic percentage in the bone marrow of the hen has attained a maximum figure among the sub-mammals rising gradually from frog to hen. This increase in lymphocytes and oxyphilic myelocyte coupled with an increase in hæmogenic tissue of the bone marrow as one ascends from frog to hen, suggests an increased lymphopoietic and oxyphilic function in the same order.



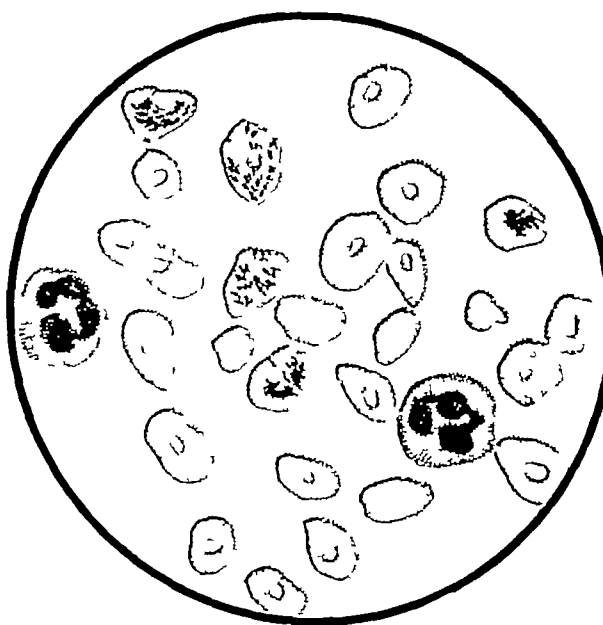
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TABLE I—*concl'd*

	<i>Blood</i> Average per cent	<i>Marrow</i> Average per cent	<i>Spleen smear</i> Average per cent
<i>Differential Counts—Hen</i>			
Polymorphonuclears	1	nil	nil
Lymphocytes	60	58	84
Mononuclears	0.75	nil	1
Transitionals	nil	nil	0.5
Myelocytes	Eosinophils	41.5	14
	Basophils	0.5	0.5

*Omnivora*

## Mouse

*Blood*—The mouse gives a very low percentage of polymorphs with large excess of lymphocytes in its blood. The average percentages for polymorphs and lymphocytes in the blood of this species are respectively 27 and 66, the former represents the lowest and the latter the highest figure amongst all mammals examined. Mononuclears number about 4 per cent, which is also a high figure for the mammals. Eosinophils are very seldom found in the blood film of the mouse, even in the course of very extensive differential count.

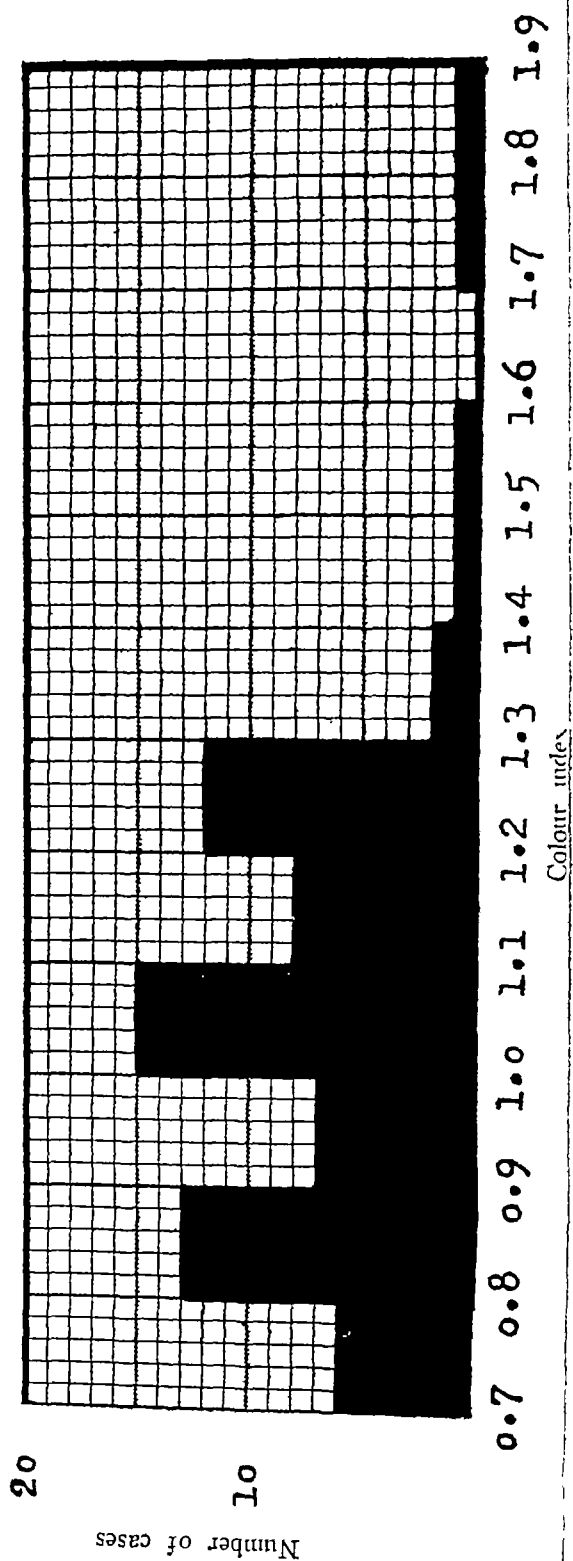
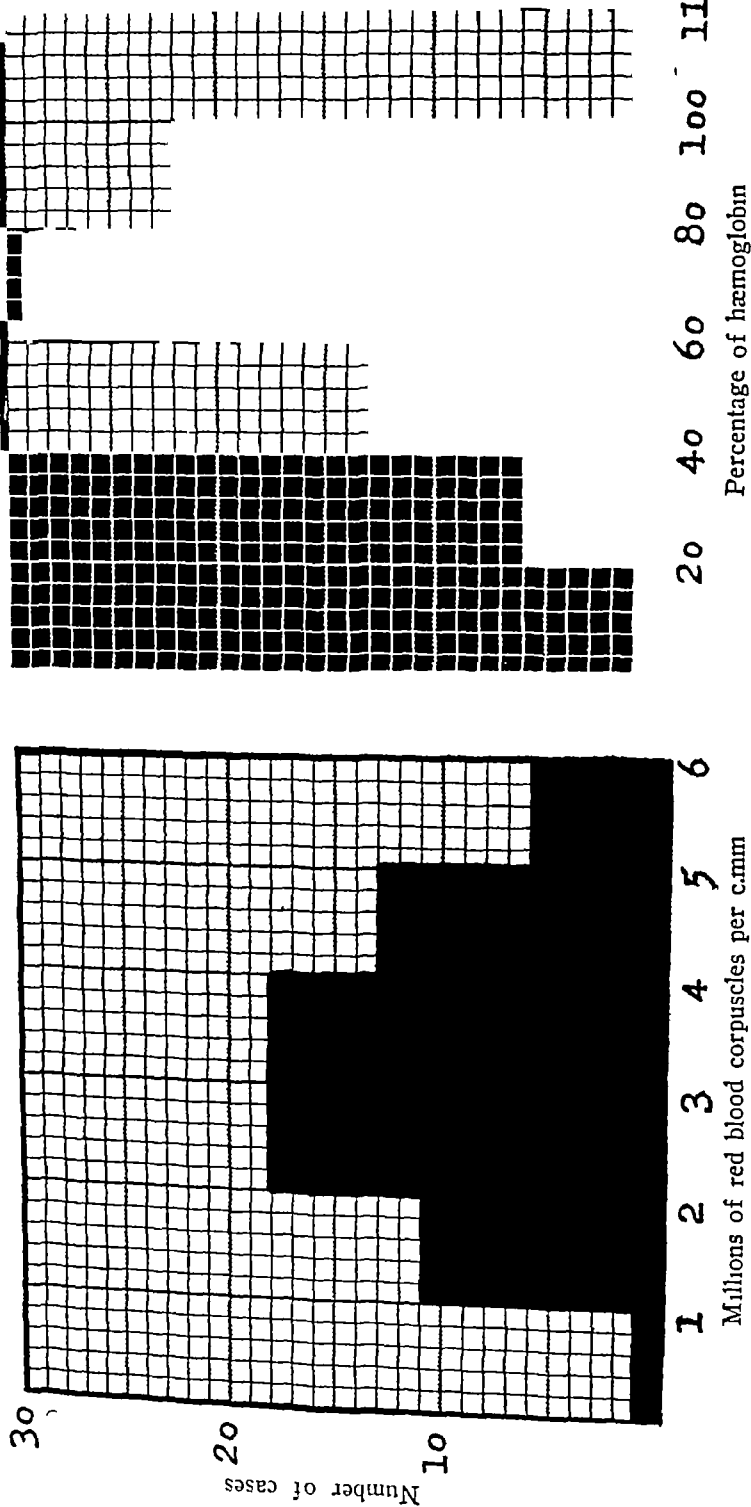
*Bone marrow*—The marrow of the mouse is richer in transitional myelocytes than the marrow of the sub-mammals, excepting the frog. But amongst the mammals examined, the lowest figure for transitional myelocytes is to be found in the mouse. Lymphocytes are less numerous in the marrow than in the blood, but the percentage in the marrow is the highest amongst all the mammals examined.

*Spleen smear*.—The spleen smear of the mouse gives about 74 per cent of lymphocytes of its total leucocytic content. This lymphocytic content of spleen is the highest lymphocytic content amongst the mammals and is less than that of the sub-mammals. Mononuclears are found to an extent of about 5 per cent and this percentage is a little higher than the monocytic percentage in blood or marrow. The spleen of the mouse contains a large number of all varieties of nucleated red blood cells, megaloblasts, normoblasts and erythroblasts.

*Spleen section*.—The spleen section of the mouse gives a distinct differentiation of Malpighian corpuscles from the pulp. The capsule and trabeculae are better developed than in the sub-mammals and contain an appreciable amount of smooth muscle. The spleen of the mouse is comparable histologically with the splenic structure of the guinea-pig. Section of the very pulpy spleen of the very young mouse often shows several megakaryocytes.

# PLATE LXIX

The Group Distributions of the Hæmoglobin, Red Blood Corpuscles, and Colour Index in 67 Cases of Sprue



Colour index

Mononuclears are a little more frequent in the blood of the rabbit than in that of the guinea-pig

*Bone marrow*—Lymphocytes, polymorphs, transitional myelocytes and oxyphil myelocytes show little difference in their percentage compared with the marrow of the guinea-pig

*Spleen smear*—Spleen smear of the rabbit contains about 67 per cent of lymphocytes of the total leucocytic content. This percentage is seen to fall as one proceeds upwards in the scale of mammals. Monocytes in the spleen of the rabbit seem to have a higher percentage than the monocytes in the spleen of the two previously described species

*Spleen section*—The spleen of the rabbit possesses a quite thick capsule and a system of fully developed trabeculae both of which contain smooth muscle to a great extent. Like the spleen of the guinea-pig it has dense and sharply stainable Malpighian corpuscles. In this species the spleen in addition to the usual cellular elements contains a rich store of big phagocytic cells in the pulp and many 'clasmatocytes' in the venous sinuses. The spleen in this species in common with the guinea-pig, goat and dog has a rich provision of 'reticulo-endothelial cells'

#### *Carnivora*

##### *Dog*

*Blood*—As compared with the herbivora the polymorphs have undergone a marked increase to an extent of about 62 per cent of the white blood cells and a correspondingly marked decrease in the lymphocytes. Transitionals and mononuclears are more abundant in the blood of this species than in the blood of the herbivorous animals, e.g., rabbit, etc

*Bone marrow*—Transitional myelocytes occur in the bone marrow of the dog to an extent of about 57 per cent which represents a very high percentage as compared with the preceding mammals examined. Oxyphil myelocytes are not very numerous in this species. Leucoblasts and megaloblasts appear to increase gradually in the red marrow of the mammals as one proceeds from mouse to dog

*Spleen smear*—Lymphocytic content in the spleen smear of dog comes down to a lower level, namely, 55.5 per cent, which represents the lowest percentage amongst mammals examined. Mononuclears are found to be quite frequent in the spleen of the dog and represent about 8 per cent of the total leucocytic content. Spleen smears contain an appreciable number of nucleated red blood cells, but this is a common occurrence in lower vertebrates. But this erythropoiesis in the spleen seems to diminish as one proceeds towards the higher animals, though no actual count of these cells was made

*Spleen section*—The dog's spleen in common with the spleen of the lower mammals, such as rabbit, goat and cat, is furnished with a thick capsule which consists mostly of plain muscle, less of fibrous and still less of elastic tissue. Malpighian follicles are sharply differentiated from the pulp. A similar differentiation has been noticed in the spleen of the adult mouse as compared with the

# PLATE LXXI

## Price-Jones' Curves of the Blood of Sprue Cases

CASE NO. 90

H. 80%  
RBC 1,440,000  
HGB 17.50  
HCT 45.5

MEAN DIAMETER

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7.50  $\mu$

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CASE NO. 95

H. 70%  
RBC 1,240,000  
HGB 15.00  
HCT 40.0

MEAN DIAMETER

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CASE NO. 98

H. 80%  
RBC 1,240,000  
HGB 15.00  
HCT 40.0

MEAN DIAMETER

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CASE NO. 99

H. 40%  
RBC 1,040,000  
HGB 10.00  
HCT 20.0

MEAN DIAMETER

6.50  $\mu$   
7.50  $\mu$

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CASE NO. 101

H. 85%  
RBC 1,240,000  
HGB 15.00  
HCT 40.0

MEAN DIAMETER

6.50  $\mu$   
7.50  $\mu$

7.50  $\mu$

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CASE NO. 102

PERNICIOUS ANEMIA

H. 24%  
RBC 1,240,000  
HGB 10.00  
HCT 20.0

MEAN DIAMETER

6.50  $\mu$   
7.50  $\mu$

7.50  $\mu$

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TABLE II

		Blood Average per cent	Marrow Average per cent	Spleen smear Average per cent
<i>Differential Counts—Mouse</i>				
Polymorphonuclears		27	21.5	16.5
Lymphocytes		66.5	54.5	74.5
Mononuclears		4	3.5	5
Transitionals		2	14	2
Myelocytes	Eosinophils	nil	6.5	1.5
	Basophils	0.5	nil	0.5
<i>Differential Counts—Guinea-pig</i>				
Polymorphonuclears		33.5	26	23.5
Lymphocytes		60	38	68.5
Mononuclears		1.5	2	4
Transitionals		1.5	20	1.5
Myelocytes	Eosinophils	2.5	13.5	2.5
	Basophils	1	0.5	nil
<i>Differential Counts—Rabbit</i>				
Polymorphonuclears		35.5	27.5	23
Lymphocytes		59	37	67
Mononuclears		2	2.5	6
Transitionals		2	20	1.5
Myelocytes	Eosinophils	1.5	13	2.5
	Basophils	nil	nil	nil
<i>Differential Counts—Dog</i>				
Polymorphonuclears		62	12	26.5
Lymphocytes		26	19	55.5
Mononuclears		3.5	3	8
Transitionals		6.5	57	6.5
Myelocytes	Eosinophils	2	8.5	3.5
	Basophils	nil	0.5	nil

# AN INVESTIGATION OF THE VALUE OF THE COMPLEMENT REACTION IN SPRUE UTILIZING *MONILIA PSILOSIS* (*ASHFORDI*) AS ANTIGEN

BY

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AND

F JASUDASAN, L M P

(Being Part XI of the Sprue Researches at the Haffkine Institute, Bombay)

[Received for publication, July 17, 1928]

A PERUSAL of the literature shows that for the last twenty-five years the fungoid theory of sprue has received considerable support by a variety of workers in different tropical countries. In later years its main advocates have been Ashford and his colleagues of the Porto Rico school who hold not only that *Monilia psilosis* (*Ashfordi*) is the true causative agent, but also that extracts of this yeast yield reliable antigens for serological reactions which are enthusiastically advocated both in clinical diagnosis and as affording an accurate index to the efficacy of treatment and the endpoint of cure.

The present series of observations deals exclusively with the serological aspects of this problem, but in former numbers of this *Journal* Mackie and Chitre have published data concerning the incidence and distribution of yeasts isolated from sprue and non-sprue cases in Bombay, as well as their classification and toxicity. Kohlbrugge (1901) first described a yeast fungus resembling *Monilia albicans* in the intestinal mucus as well as in the epithelial scales of the tongue and œsophagus of sprue cases, and during the next decade several others, including de Hann (1902), Van der Sheer (1905) and Le Dantec (1908) confirmed this. Similar organisms were sometimes found in the fæces of normal individuals. Castellani and Low (1913) demonstrated yeasts in the saliva or stools of seven out of eight cases of sprue, five different varieties being isolated. They concluded that though the monilia did not cause sprue, it might produce intestinal flatulence and frothiness of the stools. Manson-Bahr (1914) in Ceylon made a detailed study of this subject, and suggested there might be

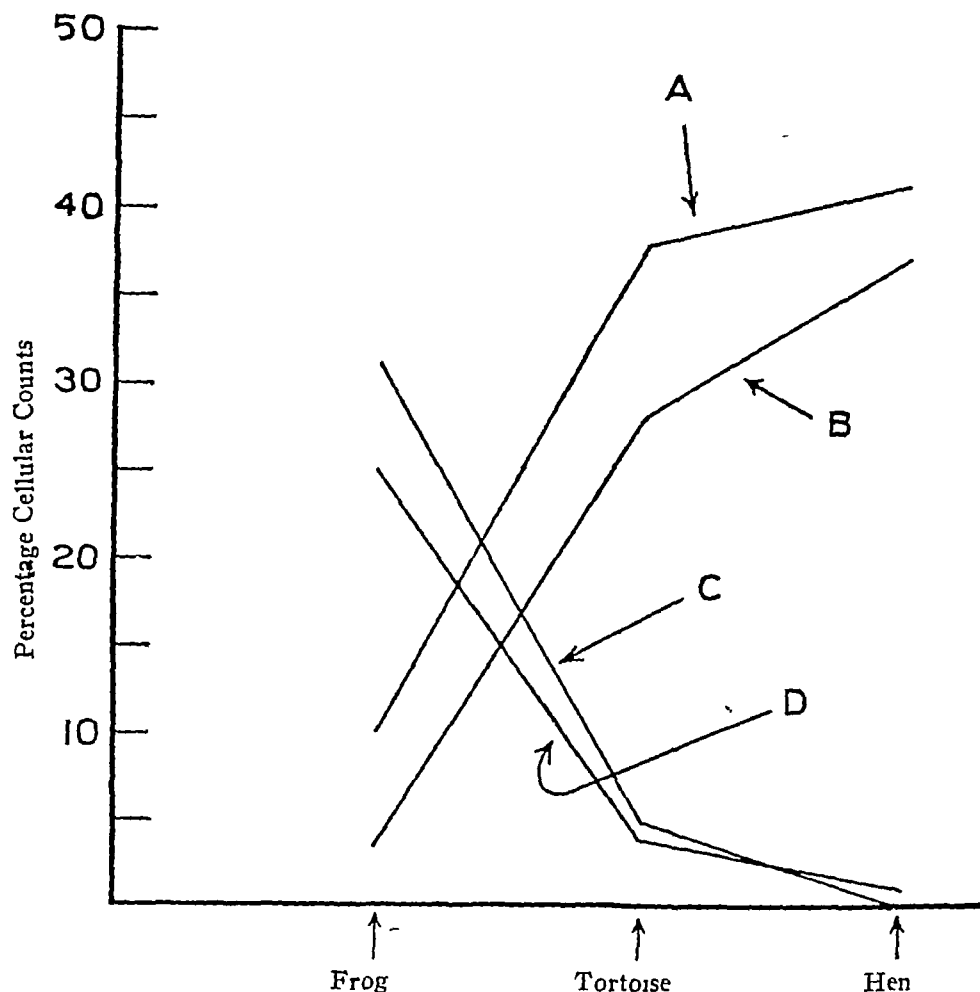


Fig 1—Showing the relation of polymorphs and oxyphils in the blood to their respective forms of myelocytes in the bone marrow

- A Oxyphil myelocytes in marrow
- B Oxyphil leucocytes in blood
- C Polymorphs and neutrophilic transitional myelocytes in marrow
- D Polymorphs in blood

and rabbit which is comparable to the gradual increase of transitional myelocytes in the bone marrow of these animals in the order described (Fig 2)

The cell count in the blood of the dog varies much from that of the rabbit or guinea-pig. In spleen smears there is a marked diminution of lymphocytes. The spleen also shows an increase in the percentage of mononuclears. Bone marrow also in regard to its cellular content presents a marked contrast with that of guinea-pig and rabbit. The percentage of transitional myelocytes is very great when compared with the percentage of transitional myelocytes in the marrow of lower animals (*cf* Figs 1 and 2). Therefore it is obvious that bone marrow

the aetiological significance of *Monilia psilosis* have been made by Hannibal and Boyd (1921), Manson-Bahr (1923) and Fairley and Mackie (1926). Thus Hannibal and Boyd found that 50 per cent of their control cases who had never suffered from sprue harboured this organism in their excreta, and Manson-Bahr failed to isolate the monilia in England. Observations at the Haffkine Institute (1924-1925) entirely failed to confirm the Porto Rico findings, and in a survey of the incidence of yeasts in human excreta *Monilia psilosis* (Ashfordi) was isolated with equal frequency in sprue patients, in non-sprue cases, and in healthy people. Furthermore, complement fixation tests on a limited number of sprue cases entirely failed to support the claims of Martinez and Michel regarding the presence of specific anti-body for *Monilia psilosis* in the peripheral blood.

### THE TECHNIQUE EMPLOYED

The sera of patients suffering from classical sprue as well as those derived from rabbits subjected by repeated intravenous injections of saline suspensions of *Monilia psilosis* (Ashfordi) were examined during the present investigation.

#### *Preparation of the Antigen*

In the preparation of the various antigenic extracts, the methods advocated by Michel (1917) were adopted. Cultures of *Monilia psilosis* were made on several slopes of Sabouraud's glucose media and incubated for four weeks. The growth was washed off with distilled water using 10 ccs for each culture, and the resulting emulsions were pooled, shaken mechanically for 2 hours and incubated for 4 days at 37°C to allow autolysation to proceed. Phenol was then added in quantities sufficient to make it up to a final strength of 0.5 per cent, and the antigen was heated for 1 hour at 58°C. Subsequently it was cultured for sterility. In addition, a similar batch of antigen was prepared, but sodium chloride as well as carbolic was added in amounts sufficient to make an approximately isotonic extract.

#### *Preliminary Standardizations*

A quantitative complement fixation technique on the lines detailed (1925) in previous publications was employed, the hæmolytic system consisting of a 3 per cent suspension of sheep's red blood corpuscles sensitized with 6 minimum hæmolytic doses (M.H.D.'s) of anti-sheep hæmolysin.

In the preliminary titration, the M.H.D. of complement was always ascertained. The anti-complementary dose of the monilia extract was also estimated by determining what dilution in unit volume would just fix 3 M.H.D.'s of complement, the range of antigenic dilution varying in a series of 12 tubes from 1/1 to 1/200.

In the final test about 1/3 of the anti-complementary dose of antigen was employed. In addition, unit volumes of the various extracts were tested over a



tional myelocytes which leads to the higher percentage of polymorphs found in the blood of these mammals, and in that the formation of lymphocytes has been transferred to specialized lymphoid tissue which is capable of producing lymphocytes when suitably stimulated (Fig 3)

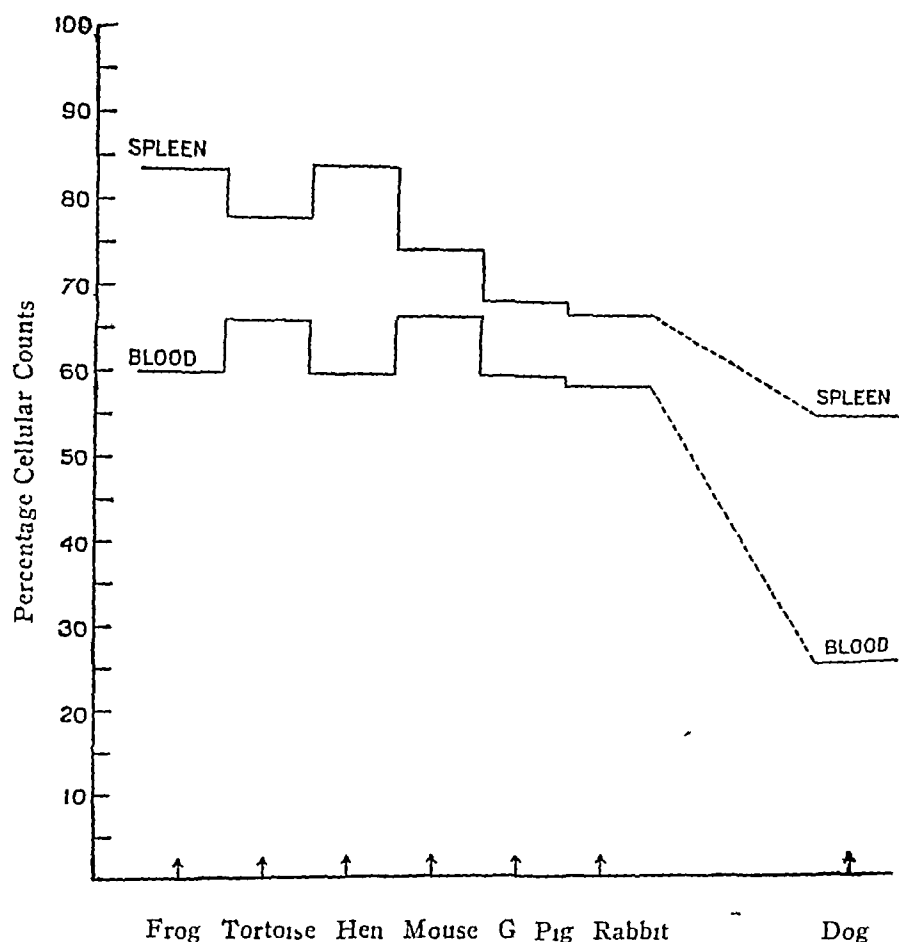


Fig 3—Showing the relation in vertebrates (mammals and sub-mammals) of lymphocytes in the spleen and the blood

*On Neutrophilic Leucocytes of Blood*—In the post-natal life neutrophils are mostly formed by the red marrow of bones and partly by the spleen

Blood of reptilia and aves is remarkably poor in neutrophilic cells. This fact is intimately related with the corresponding paucity of transitional myelocytes in the red marrow of the sub-mammals in question (Figs 1 and 4). The gradual decrease of polymorphs in the blood with the accompanying decrease of transitional myelocytes in red marrow can be well traced from frog to hen through tortoise (Fig 1) whereas in the mouse, guinea-pig, rabbit and dog the polymorphonuclear leucocytes in the blood become gradually higher and higher in percentage and the

Of the 18 sera investigated by this particular method of complement fixation 9 were cases of sprue, 5 were derived from healthy adults and 4 from syphilitics with positive Wassermann reactions. None of the normal or luetic sera reacted positively. Seven of the sprue sera yielded completely negative results with each of the three monilia extracts. The remaining two sera showed with two out of the three monilia extracts an inhibition of hæmolysis with 3 M H D's of complement over a wide range of antigenic dilutions associated, however, with distinct zonal effects. In the case of the third monilia extract both sera yielded negative reactions, and on re-investigation by Technique B similar negative results were obtained. Zonal effects were not observed in the case of any of the other sera tested and we attribute these atypical results rather to a non-specific adsorption of complement in a complex colloidal system than to a specific antigen-antibody reaction.

#### *Technique B*

The extracts investigated by this technique included strains of *Monilia psilosis* (Ashfordi) kindly supplied by Colonel Bailey Ashford to the Haffkine Institute, one strain having been isolated from the fæces, and the other from the tongue of sprue cases.

In all sixteen sera were examined, eight being derived from sprue cases and eight from patients who had never suffered from this disease. In the latter group were four syphilitics with positive Wassermann reactions.

Fourteen of the sixteen sera, including all those obtained from sprue cases, gave negative results. The remaining two which were Wassermann positive showed some inhibition of hæmolysis of limited range giving a type of reaction which in ordinary nomenclature would be described as a partial positive +, signifying the fixation of 3 M H D's of complement. Such a finding calls for little comment as it is well known that saline extracts of animal tissue, metazoan parasites or bacteria are liable to react with strongly positive syphilitic sera in this fashion.

#### *Commentary on the Results*

The combined results of the two methods are included in the following protocol (Protocol I). It will be seen that the sera of only 2 out of 17 cases

#### PROTOCOL I

*Complement fixation reaction in sprue sera utilizing Monilia psilosis (Ashfordi) as antigen*

Method	SPRUE SERA		CONTROL SERA	
	Number examined	Positive reaction	Number examined	Positive reaction
Technique A	9	2	9	2
Technique B	8	0	9	2*
TOTAL	17	2	18	0

\* Both these were syphilitics with positive Wassermann reactions

Polymorphonuclear leucocytes mainly come from the transitional myelocytes of bone marrow in the animals like guinea-pig, rabbit and dog, etc. But during certain periods of life this production of polymorphs may be thrown upon the spleen according to the special needs of the organism in the environment. In the sub-mammals as the spleen is not of a very high order of organization, bone marrow alone might serve the purpose of producing the few neutrophils that are met with in these lower animals.

*On Mononuclear Leucocytes of Blood*—The monocytic content of the blood shows a fall as one proceeds from frog to hen through tortoise (Fig 5). This fall does not seem to possess any distinct relation to the productiveness of the same type of cells on the part of bone marrow or the spleen because neither of these tissues shows any concurrent increase or decrease. It is possible that both the spleen and bone marrow participate in the formation of mononuclears.

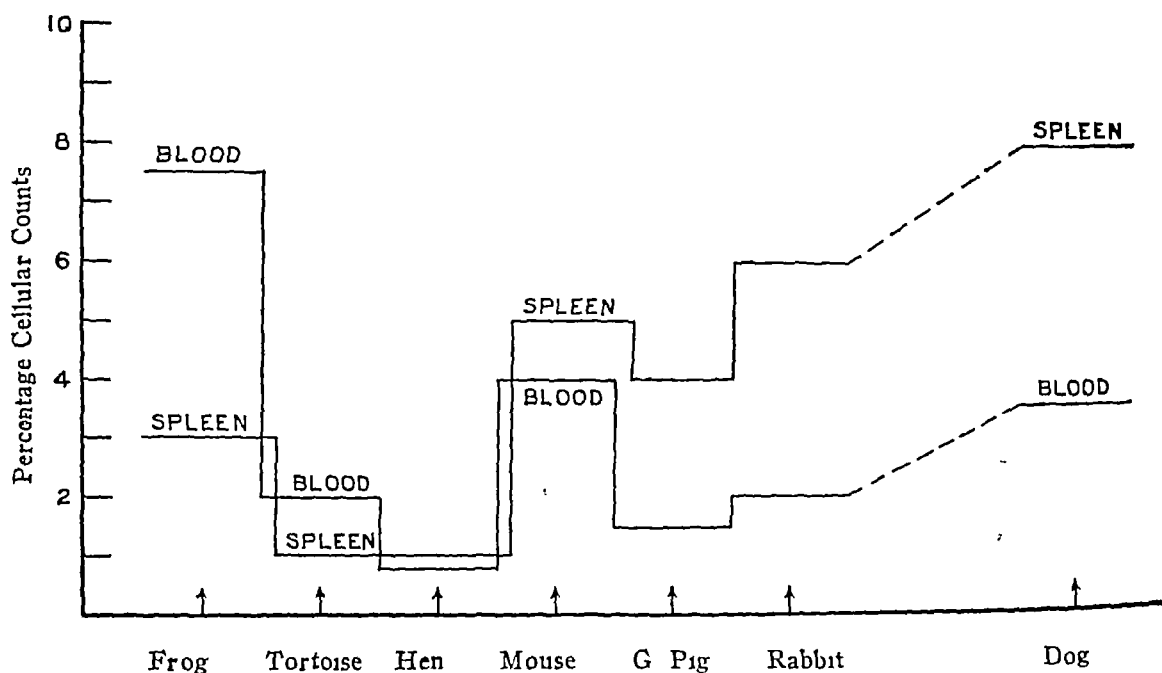


Fig 5—Showing the relation in mammals and sub-mammals of mononuclears in the spleen to those in the blood

In the mammals we have noticed that the monocytic content of the spleen has a direct bearing upon the monocytic content of the blood, this is seen from a consideration of Table II which would indicate that the formation of mononuclears in mammals is a function that mostly concerns the spleen (Fig 5).

*On Transitional Leucocytes of Blood*—A careful examination of the tabulated results of the cell counts shows that as the transitional myelocytes increase in the marrow there is a definite increase of transitional leucocytes in the blood and spleen of the dog and a marked increase in the neutrophils of the blood. As has

sera were de-complemented by heating for 20 minutes at 55.5°C. The latter procedure in the case of certain animals has been criticized by Kolmer, Rule and Tristi (1920) who record that heating the sera of normal rabbits, dogs and mules at 55°C for 15 to 30 minutes greatly increases their property of yielding non-specific complement fixation reactions with various antigens of tissue extracts, bacteria and the like. We found that the sera of some of our control rabbits occasionally reacted positively with monilia extracts prior to inoculation, when Technique A and the standard 3 M H D's of complement was employed. The tendency to pseudo-positive reaction, however, was greatly decreased by increasing the complement to 4 M H D's per unit volume and unless this is done the method is unreliable for these animals.

Undoubtedly Technique B is the more suitable for serological work on rabbits and naturally no finding is of value unless the animal has yielded a negative serological test prior to inoculation.

The actual results obtained in a series of 14 rabbits injected intravenously with emulsions of *Monilia psilosis* (Ashfordi) are detailed in Protocol II. It will be seen that the sera of 12 out of the 14 animals fixed large quantities of complement (15 to 24 M H D's) as a result of the injections, while the other two deviated about 5 M H D's. It is interesting to note the high grade response in the sera of rabbit No. 37 on the 10th day following inoculation. The control sera were all negative. Results such as these show the facility with which anti-body can be produced by experimental inoculation with *Monilia psilosis* (Ashfordi), and also the satisfactory nature of our monilia extracts when used as antigens for the complement fixation test. Identical antigens yielded negative results with human sera from sprue cases and our serological findings fail to confirm the claims of Ashford, Martinez and Michel regarding the presence of monilia anti-body in the peripheral blood in this disease.

#### SUMMARY AND CONCLUSIONS

1 Rabbits inoculated intravenously with saline suspensions of *Monilia psilosis* (Ashfordi) yield sera of high grade titre when tested *in vitro* by means of the complement fixation reaction using aqueous extracts of the monilia as antigen.

2 The sera of 12 out of 14 inoculated rabbits fixed from 15 to 24 M H D's of complement some 10 to 32 days after the first injection, while the sera of the other two fixed about 5 M H D's.

3 Identical antigens when tested against human sera derived from sprue patients failed to show positive reactions in 15 out of 17 cases tested.

4 In the remaining 2 cases the reaction, owing to its zonal nature, was regarded as a non-specific adsorption rather than a true antigen-antibody phenomenon.

5 No confirmation of the claims of Ashford, Martinez and Michel regarding the presence of circulating monilia anti-body in the sera of sprue cases was obtained.

erythrogenic function of the spleen is gradually shared by the bone marrow throughout the sub-mammalian series, for example, bone marrow of the frog is the least erythrogenic, whereas bone marrow of the hen is the most erythrogenic, conversely the spleen of the frog is most actively erythropoietic, the spleen of the tortoise a little less so and that of the hen least

The lymphocytic content of the spleen is one of the main controlling factors of the lymphocytic content of the blood. The low lymphocytic content of blood in the higher mammals is an indication of the better organization of the lymphopoietic apparatus, namely, the Malpighian corpuscles in the spleen. With the increased organisation of Malpighian follicles, there is a diminution of lymphocytes in the pulp together with the increased differentiation of leucopoietic activity as has been evidenced from the high content of polymorphs and monocytes in the spleen of the dog. The proportion of mononuclears and granulocytes to lymphocytes in the pulpy spleen of the lower vertebrates is much less than the proportion of these cells in the spleen of the dog.

This high cell ratio in the spleen is an index of the activity of the organ both in the direction of leucopoiesis and storage. Mononuclears of the mammalian blood for their origin find a much richer source in the spleen than in the marrow.

Throughout the mammalian series, a gradual increase in the percentage of polymorphs in the blood is seen to be associated with a gradual increase of the total percentage of polymorphs and neutrophilic transitional myelocytes in the bone marrow. On the other hand, the paucity of the neutrophilic transitional myelocytes in the marrow of aves and reptilia is correlated with a marked dearth of the neutrophil leucocytes in the circulating blood. Bone marrow thus stands prominent as the main source for the supply of polymorphs.

Transitional leucocytes of the blood have their origin direct from the myelocytes. The latter type of cells in marrow shows a varying number of granules with a varying staining capacity in accordance with the age of the individual cell. It is probable that some of them undergo chemical changes in their cytoplasmic granules and enter the circulation as transitional leucocytes. But they represent neither a stage in the development of large mononuclears found in the blood, nor do they have any immediate parentage common both to themselves and to mononuclears.

Oxyphil leucocytes of the blood vary directly with the oxyphil myelocytes in the marrow of the vertebrates examined, a relation which is further demonstrated in helminthic infections.

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TABLE I

*Differential Counts of Blood, Bone-marrow Films and Spleen Smear of the Control Puppies*

		Blood Average percentage	Bone marrow Average percentage	Spleen smear Average percentage.
<i>Puppy No 1</i>	Polymorphs	57	11	18
	Lymphocytes	34	25	67
			Leucoblasts 12	
	Mononuclears	25	3.75	4
				Splenocytes 25
	Transitionals	3.5	Myelocytes { 10 38.25	4
	Eosinophils	3		4.5
<i>Puppy No 2</i>	Basophils	nil	nil	nil
	Polymorphs	57.5	7.5	17
	Lymphocytes	31	21	68
			Leucoblasts 19	
	Mononuclears	3.5	3	3
				Splenocytes 35
	Transitionals	6	Myelocytes { 44 5.5	25
<i>Puppy No 5</i>	Eosinophils	2		6
	Basophils	nil	nil	nil
	Polymorphs	56	7	15.5
	Lymphocytes	38	17	68
			Leucoblasts 14	
	Mononuclears	2.5	5	6
				Splenocytes 35
	Transitionals	2.5	Myelocytes { 48 9	2
	Eosinophils	1		5
	Basophils	nil	nil	nil

- 5 Examination of cellular elements in smears of spleen pulp
- 6 Examination of cellular elements in spleen sections

## NOTE ON OBSERVATIONS MADE ON BLOOD, BONE MARROW AND SPLEEN

*Amphibians**Frog*

*Blood*—Lymphocytes come first in order of frequency in the circulating blood. Smaller lymphocytes should be carefully examined in order to differentiate them from the large sized blood platelets. Polymorphs form 25 per cent of the total leucocytes. With the exception of a slight incomplete lobulation of the nucleus they resemble the polymorphs in mammalian blood. In sub-mammals, we have found 7 to 8 per cent of a type of leucocyte which is difficult of classification as polymorphs or lymphocytes. In the amphibians and reptiles we have classed this type as mononuclears, owing to their resemblance to the mononuclears in mammalian blood in respect to their non-granular cytoplasm. As it is very difficult to differentiate the various cell types in the sub-mammals, it would not seem out of place to note that the blood of certain frogs shows a considerable number of cells with a very inconspicuous nucleus surrounded by large number of coarse amphophil granules. With regard to these coarsely granular bodies it still remains to be shown whether they are some special form of granulocyte or are mere artifacts.

*Bone marrow*—The percentage of lymphocytes in the bone marrow of the frog, though as high as 53, is a little less than that of the lymphocytes in the blood. Transitional myelocytes, and by that we mean neutrophilic transitional myelocytes, as opposed to oxyphilic and basophilic, in the bone marrow have a lower percentage than polymorphs in the blood, whereas oxyphil myelocytes have a much higher percentage than that of oxyphil leucocytes in the blood. The bone marrow of frogs presents almost a negligible number of megalo-, normo- and erythro-blasts.

*Spleen smear*—In frog the differential count of the spleen smear preparations reveals the percentage of lymphocytes to be as high as 83.5. Neutrophils are present to an extent of only about 11.5 per cent of the total leucocytes of the organ. Regarding the formation of nucleated red blood cells, it is found that in frogs the spleen is far more active than the marrow. There are very few megaloblasts in the bone marrow of the frog.

*Spleen section*—The frog's spleen consists essentially of pulp tissue. It has a very thin fibro-muscular capsule which sends very few visible trabeculae into the interior of the section. Malpighian corpuscles as such are not distinguishable from the pulp. The continuity of the uniform lymphocytic distribution in the section is here and there interrupted by the presence of venous sinuses filled with erythrocytes. In an irrigated spleen section, the reticulum of the spleen and a few amœboid phagocytic splenic cells are observed. The venous sinuses in the frog's spleen are lined by clasmatoocytes of Ranvier which, however, show no continuity from one cell to another.



ammon citras It would therefore seem that, as far as leucopoiesis is concerned, iron does not act as a stimulant of lymphoid tissue

From a consideration of the cell counts, it would appear that in puppy No 8 there is a marked increase of polymorphs in the spleen with a corresponding increase of polymorphs in the blood Another similar fact is noticed in the cell counts of puppy No 7, where with the increase of eosinophils in the blood there is a corresponding increase of eosinophilic myelocytes in the bone marrow But as these facts are not common to all of the puppies treated with iron salt, we are inclined to ascribe the increase as due to some infection rather than to any specific action of the iron salt

There is a faint indication of an increased maturation of megaloblasts in the red marrow of the ribs

On comparing the spleen section and the spleen smear preparation of the iron-fed puppies with those of controls, a definite increase of nucleated red blood-corpuscles is found in the case of the former An increased proliferation of the phagocytic cells can be seen by their frequent presence in the pulp and sinuses laden with yellowish-green granules

*On Reticulocytes*—That the increase of reticulocytes in the circulation is an index of the increased activity of the erythrogenic organs has almost been accepted Reticulocytes, more strictly speaking 'reticular normocytes,' are now regarded as the young red blood-cells and they appear in numbers in the blood stream when there is some stimulus action on the bone marrow and other red blood-cell forming organs The same fact has lately been reported by various clinical observers in connection with the treatment of pernicious anæmia by liver feeding

In our work with the iron treatment of the experimental animals, notes of reticulocyte counts were made in some of the puppies, the results are shown in Table III A consideration of the table shows that the experimental puppies Nos 10, 11, 12 and 13 undergoing iron treatment for a fortnight showed a definite increase in their reticulocyte counts The increase was more marked from the end of the second week to the end of the third week and during the fourth week the reticulocyte percentage gradually went down, in some of them even below the initial percentage Puppy No 10 alone continued to maintain the high reticulocyte count even towards the end of the fourth week It should be noted that iron administration was stopped on the 16th day in every case though the effect on the reticulocyte percentage continued to be markedly potent till the end of the third week

Puppy No 16, which seemed to be an extraordinarily healthy dog with a low initial reticulocyte count, did not show even the slightest increase of reticulocyte percentage as a result of the continued intravenous administration of ferri et ammon citras in 0.25 gramme doses

Control puppies Nos 14 and 15 did not show any marked variation in their reticulocyte percentages during the same time Thus, from the above results, it appears that ferri et ammon citras in moderate doses both by intravenous and

*Spleen smear*—The spleen smear of the hen contains approximately the same high percentage of lymphocytes as are found in the spleen of the frog and tortoise. Polymorphs and transitionals are very rare, and eosinophils are rather less frequent in the spleen than in blood.

*Spleen section*—The spleen of the hen is also a pulpy one. It has a thick fibro-muscular capsule containing a little elastic tissue. The extent of smooth muscle present in the capsule of the spleen in the hen is by far greater than that met with in the capsule of the frog and tortoise. The spleen section presents a very compact appearance owing to its high content of lymphocytes. The tissue of the Malpighian follicle is not different from that of the pulp but it is capable of recognition as lymphoid tissue in virtue of the fact that it is surrounded by a thin fibrous sheath.

The increase of eosinophils in blood from frog to hen through tortoise, with the accompanying fall of polymorphs in the same order seem to indicate that there may be some relationship in sub-mammals between polymorphs and eosinophils. Since the percentage of eosinophils is very high in tortoise and hen, it would seem that they carry out some function common both to eosinophils and polymorphonuclears in their sub-mammalian form of life.

The above observations are numerically shown in Table I.

TABLE I

	Blood Average per cent	Marrow Average per cent	Spleen smear Average per cent
<i>Differential Counts—Frog</i>			
Polymorphonuclears	25	14	11.5
Lymphocytes	60	53.5	83.5
Mononuclears	7.5	4.5	3
Transitionals	3.5	17	2
Myelocytes {	Eosinophils	3.5	nil
	Basophils	0.5	nil
<i>Differential Counts—Tortoise</i>			
Polymorphonuclears	4	3	nil
Lymphocytes	66	56	78
Mononuclears	2	1	1
Transitionals	nil	2	nil
Myelocytes {	Eosinophils	28	20.5
	Basophils	nil	0.5

TABLE III  
*Reticulocyte Counts in the Blood of the Experimental Puppies*

Puppy No	Initial average percentage	PERIOD WITH IRON SALT						PERIOD WITH NO IRON SALT			
		3rd day average percentage	6th day average percentage	9th day average percentage	12th day average percentage	15th day average percentage	16th day average percentage	18th day average percentage	20th day average percentage	26th day average percentage	28th day average percentage
10	1.75	3.0	6.0	4.5	2.5	5.0	4.5	6.5	5.5	6.0	5.5
11	3.0	3.5	4.0	6.0	7.5	9.5	12.0	10.5	10.0	0.5	0.25
12	2.5	3.0	2.5	5.0	5.5	5.5	4.5	6.5	7.0	2.5	2.0
13	2.5	3.0	0.75	1.5	3.0	4.0	3.5	6.0	6.25	1.25	0.75
16	0.75	0.75	0.25	0.2	0.2	0.25	0.2	0.2			

*Reticulocyte Counts in the Blood of the Control Puppies*

Puppy No	1st day average percentage.	3rd day average percentage.	6th day average percentage	9th day average percentage	12th day average percentage	15th day average percentage	16th day average percentage	18th day average percentage
14	4.25	3.0	4.0	2.5	3.5	4.0	3.0	3.5
15	3.5	3.5	4.5	3.0	2.5	1.5	1.75	2.25

*Herbivora*

## Guinea-pig

It is difficult to arrive at correct differential counts in animals like the guinea-pig, rabbit and dog, as nearly 40 per cent of them are subject to some sort of infection and give variable differential counts, often with a very high eosinophilic or neutrophilic content of the blood

*Blood*—Polymorphs are present to an extent of about 33·5 per cent in this species. This percentage is a little higher than that of the mouse. Lymphocytes are about 60 per cent and in a little less percentage in guinea-pig's blood than in the blood of the mouse. Of the total white cell count, mononuclears, transitionals and eosinophils number 1·5, 1·5 and 2·5 per cent respectively

*Bone marrow*—The bone marrow of the guinea-pig is a little richer in transitional myelocytes and a little poorer in lymphocytes than that of the mouse. In comparison with the bone marrow of the mouse, oxyphil myelocytes in the marrow of this species are found to be increased a little with an accompanying increase of oxyphil cells in the blood. Myeloblasts and megaloblasts are more frequent in the marrow of these mammals than has been observed in the sub-mammals

*Spleen smear*—The lymphocytic content in the spleen smear of the guinea-pig is less than that of the mouse and this fall of the lymphocytic content is continuous in all animals above the mouse

Large mononuclears are found to the extent of nearly 4 per cent of the total leucocytes present in the spleen of the guinea-pig. Polymorphs occur in the spleen in a much less percentage than they do in the general circulation

*Spleen section*—The spleen of the guinea-pig has a well developed fibromuscular capsule from which many stout trabeculae containing mostly plain muscle project into the spleen pulp. Here Malpighian follicles are quite sharp and easy of distinction from the pulp. The spleen pulp is extensively traversed by arteries, venules, and venous sinuses and the pulp tissue just beneath the capsule is more dense than the interior, while it contains a large number of disintegrated normal corpuscles and nucleated red blood corpuscle with many lymphocytes and neutrophils. Malpighian follicles do not show any speciality in composition, their usual content being lymphocytes and a pair of small arteries. The presence of venous sinuses in close relation to the Malpighian corpuscles has been noticed in the spleen of the guinea-pig. The venous sinuses externally present a very thin fibrous layer which contains a few endothelial leucocytes. In some of the guinea-pigs, spleen venous sinuses contain large numbers of neutrophils and lymphocytes together with one or two monocytes impregnated with some sort of pigment. It is possible that these sinuses are filled with cells formed by the pulp as well as by the endothelial cells of the sinus

*Herbivora*

## Rabbit

*Blood*—Rabbit's blood contains almost the same low percentage of polymorphs and high percentage of lymphocytes as the blood of the guinea-pig

chamber gently for 15 minutes, and then strongly for 30 minutes. At the end of this time the walls of the flask are free from charred material and its contents consist of a brown homogeneous fluid. This is allowed to cool and then diluted with an equal volume of water and transferred to a 50 c.c. stoppered graduated flask. A rise of temperature occurs on diluting and washing the fluid into the graduated flask. When room temperature is again reached, the contents of the flask are made up to the mark. A certain amount of a light, flocculent precipitate forms on adding water to the acid digestion fluid. The contents of the flask are therefore thoroughly shaken and 5 c.c.s. are withdrawn immediately and placed in a Pyrex tube, to which 1 c.c. of 'perhydrol' is added. The tube is inclined as in the previous method and the oxidation with 'perhydrol' there outlined is followed. If much iron is present, the final solution has a slight yellow colour after oxidation which almost entirely disappears on cooling.

After cooling, the solution is transferred to a 50 c.c. graduated flask and acetone and thiocyanate solution added as before, a standard for comparison being also prepared as already described.

In the case of organs containing more than the normal amount of iron, 0.5 gramme should be taken instead of 1 gramme and it may be found necessary to prepare a stronger standard solution for colorimetric comparison.

TABLE IV  
*Average Iron Contents*

	Weight in kilogrammes varying between	Blood Average percentage in milligrammes per 100 c.c.	Number of samples analysed
1 Healthy adult dogs	9 to 12	51.4	9
2 Diabetic adult dogs	9 to 12	35.12	6
3 Puppies	5 to 9	41.25	15

*Average percentage of Dry Matter in Fresh Tissue after thorough desiccation in steam oven*

1 Spleen	22.5
2 Liver	27.15
3 Kidney	24.8

*Average Iron Contents of Spleen, Liver and Kidney in Puppies*

Tissue	Average percentage in milligrammes per 100 grammes of dried tissue	Number of samples analysed
1 (a) Spleen, dried	71.5	7
(b) Spleen, with traces of blood	138.0	3
2 Liver, dried	49.7	4
3 Kidney, dried	44.9	4

young mouse and sub-mammals, but such differentiation must be correlated with changes in the cellular elements in arriving at any conclusion as to evolution of function. Regarding the pulp in the spleen of the dog we are convinced by actual counts that the lymphocytic content is much less than that of the lower vertebrates. The pulp of the spleen of the dog is composed of cell elements, such as lymphocytes, neutrophils, amoeboid phagocytic splenic cells, ordinary erythrocytes and normoblasts, while all pulpy spleens of the very low vertebrates like the frog contain a large number of lymphocytes, erythrocytes and erythroblasts and less of granulocytes. In other words, the proportion of mononuclears and granulocytes to lymphocytes in the spleen pulp of the lower vertebrates, namely 1/5, is much less than the proportion of these cells in the spleen pulp of an animal such as the dog, where it is 1/12 (*see* Table II). The dog has a higher granulocytic and monocytic content both in the spleen and in the blood than lower forms possess, and by virtue of this high ratio of granulocyte to lymphocyte, the dog's spleen may be regarded as functionally more developed than the spleen of the mouse or guinea-pig or rabbit where the proportion varies only from 1/3.5 to 1/2. This would also support the idea that a lower lymphocytic count is associated with a better functional differentiation of the Malpighian corpuscles which would also point to the fact that the lymphoid tissue of the spleen had become better organized for the immediate production of lymphocytes upon demand by the organism.

#### DISCUSSION

##### *The Significance of the Spleen and Bone Marrow as Members of the Erythropoietic and Leucopoietic Systems in the Vertebrates*

*Sub-mammals*—Since in the frog the red marrow is very feebly developed the spleen has, in addition to its lymphopoietic function, to meet the demand of the organism for red blood cells. The yellow marrow is associated with a very high percentage of fat and thus the total hæmogenic element, being much reduced, the formation of polymorphs and eosinophils is limited, thus accounting for the small number of polymorphs and eosinophils found in the circulation. While the frog has a small amount of bone marrow, it may be assumed in view of the small number of polymorphs and eosinophils in its blood, that this amount of marrow is ample for the granulocytic needs of the animal.

In the tortoise the bone marrow is a little more developed than in the frog and in the hen a still further development is to be seen. With this fact in view, we note, first, a gradual increase of oxyphil myelocytes in the marrow and oxyphil leucocytes in the blood from frog to hen through tortoise, and, secondly, a disappearance of polymorphs from the blood and of transitional myelocytes from the marrow. This disappearance which is almost complete in the hen is difficult of explanation, but it would indicate that the leucopoietic function of the marrow as far as transitionals and polymorphs are concerned becomes less and less as we proceed from frog to hen.

TABLE V—contd  
*Experimental*

Puppy No		Spleen	Liver	Kidney	INITIAL		FINAL *	
					Blood	Plasma	Blood	Plasma
3	Received intravenous injection of ferri ammon. citras for two weeks, 0·125 gramme daily One raw egg was also given in addition to ordinary diet.	(1) 124·0	(1) 160·0	(1) 56·3	(1) 36·2		(1) 47·4	(1) 4·4
12	Do Received intravenously 0·125 gramme of ferri ammon citras daily for 15 days One raw egg was also given every day in addition to ordinary diet	(11) 121·0	(11) 162·0	(11) 57·4	(11) 35·8 (1) 42·5		(11) 46·5 (1) 42·8	(11) 3·6
8	Do Received intravenous injection of ferri ammon citras for 15 days, 0·25 gramme a day One raw egg was also given daily in addition to ordinary diet	(1) 195·2	(1) 177·8	(1) 68·3	(11) 42·1 (1) 42·7		(11) 43·9 (1) 40·8	(1) 3·9
11	Do Received intravenous injection of ferri ammon citras for 15 days, 0·25 gramme a day One raw egg was also given daily in addition to ordinary diet	(11) 192·8	(11) 175·6	(11) 68·0	(11) 43·2 (1) 42·5 (11) 43·4		(11) 40·1 (1) 44·1	(11) 4·7

\* For the final iron content of the blood of every experimental puppy, estimation was carried out not before full 36 hours had passed after the last administration of ferri ammon citras, whether orally or intravenously

The spleen in view of these facts has not progressed much in the differentiation of pulp and Malpighian follicles and the differential counts of the spleen smear preparations in these sub-mammals do not show the least sign or differential leucopoiesis. But it is noteworthy that in the spleen of frog, tortoise and hen we do find an almost constant and high percentage of lymphocytes. This constancy in lymphocytic percentage points to the fact that the spleen in sub-mammals has very little to do with the differential leucopoietic activity, it continues through the series to produce lymphocytes and nucleated red blood corpuscles to the blood stream.

Another interesting feature in the erythropoietic function in these sub-mammals is, that as we proceed from frog to hen through tortoise, the spleen throws back its erythropoietic function little by little on the gradually developing bone marrow. This is shown by the fact that the spleen of the tortoise contains fewer megaloblasts than does the spleen of the frog, and similarly the spleen of the hen contains fewer megaloblasts than that of the tortoise. Therefore it is not impossible to think of a similar gradual evolution of the erythropoietic function on the part of marrow throughout the lower animals. This charge is completely made over to the bone marrow when the skeletal system is sufficiently developed to meet the requirements of the organism for red blood cells, for example, in human beings this function of the formation of red blood cells is fully transferred to the bone marrow in post-natal life and the spleen becomes quite free from megaloblasts unless any pathological condition supervenes.

*Mammals*—On looking at the differential counts of blood, it will be seen that at the stage of the rabbit in the mammals, the lymphocytic content has not undergone much decrease and the percentage in the blood is still about 60. But the differential counts of spleen smears give an appreciable and steady fall from one animal to the next higher animal in the scale of evolution. There can be no doubt that the spleen during these stages of evolution has been constantly decreasing its lymphocytic content and increasing within its pulp the number of neutrophilic and monocytic cells. Evidences for this gradual phylogenetic increase of cellular elements in the spleen is given in Table II. It would seem that the spleen during this process of evolution develops special areas for the production of lymphocytes, and thus we have the increasing differentiation of Malpighian follicles in the spleen of the mouse, guinea-pig and rabbit, etc.

Our experimental animals in sub-mammals ended with the hen, which gives a very high percentage of eosinophils and eosinophilic myelocytes in the blood and marrow respectively (Fig 1). The mouse was chosen for the lowest available specimen amongst the mammals, it shows a dearth of oxyphil leucocytes in blood and gives a very low percentage of oxyphil myelocytes and in comparison with other mammals has a comparatively low percentage of transitional myelocytes and polymorphs in its marrow (Fig 2). In the mouse, a little better differentiation in the function of the bone marrow in the formation of transitional myelocytes is noticed as compared with sub-mammalian forms. Further is a gradual increase in the percentage of neutrophils in the blood of the mouse, guinea-pig,



The average iron content of the spleen containing traces of blood is much higher than that of the spleen properly bled and washed with distilled water. The great variation of the iron content amongst unwashed spleen samples arises from the varying blood content of the organ, due to its highly vascular nature.

*Discussion of Results*—A careful observation of Table V reveals that amongst the controls, puppies Nos 1 and 9 have suffered a slight loss in the iron content of their blood after the observation period of a fortnight, puppy No 15 gained a little and puppies Nos 2 and 14 maintained the level of iron content almost constant after the same length of observation period. Thus the controls vary in the iron content of their blood within the limits of 1 to 2.5 mg below or above the initial level.

Regarding the experimental puppies in the first set, puppies Nos 10 and 13 received by mouth ferri et ammon citras 0.125 gramme daily with egg for 15 days, there was no increase in the final iron content of blood in case of puppy No 10 and there was hardly a gain of 1 mg in case of puppy No 13. In the second set, puppies Nos 6 and 7 received orally ferri et ammon citras 0.25 gramme a day, the former for three weeks and the latter for two weeks, there was a slight fall of the iron content of blood from the initial level in case of puppy No 6 and a slight gain in the final iron content in case of puppy No 7. In the third set, puppies Nos 3 and 12 received the iron salt 0.125 gramme a day intravenously for a fortnight, there was an appreciable increase in iron content of the blood of the former and there was practically no increase in case of the latter. But much weight should not be attached to the increase in iron content of the blood in case of puppy No 3, because the blood sample for iron estimation was taken just after the administration of ferri ammon citras.

In the last set, puppies Nos 8 and 11 received intravenously double the dose of the drug for 15 days, the final iron content of the blood of the former showed a slight fall while that of the latter showed but little variation from the initial level.

With these facts in view, it is difficult to ascribe to iron salt (ferri et ammon citras), whether given orally or intravenously, any activity in increasing the iron content of the circulating blood of the experimental puppies, where in common with the controls there was a variation of not more than 1 to 2.5 mg either below or above the initial level of iron content of the blood. Thus the experimental puppies receiving iron salt behaved exactly like the controls as far as their content of blood iron is concerned.

With regard to the spleen, liver and kidney as depôts of iron in normal dogs, it would seem that the comparative degree in which these organs store iron is as in the order mentioned. It should be noted that this iron content is per gramme of dried tissue and not the absolute quantity in the whole organ.

It is seen after iron treatment that in the puppies Nos 3 and 6 the iron content of liver per 100 grammes is greater than that of spleen, whereas in the puppies Nos 7 and 8 the iron content of spleen is greater than that of liver.

also in the dog has passed through several stages of evolution so far as its capacity for giving rise to varieties of leucocytes is concerned It can be said that those leucoblasts that produced oxyphilic myelocytes and transitional myelocytes at the

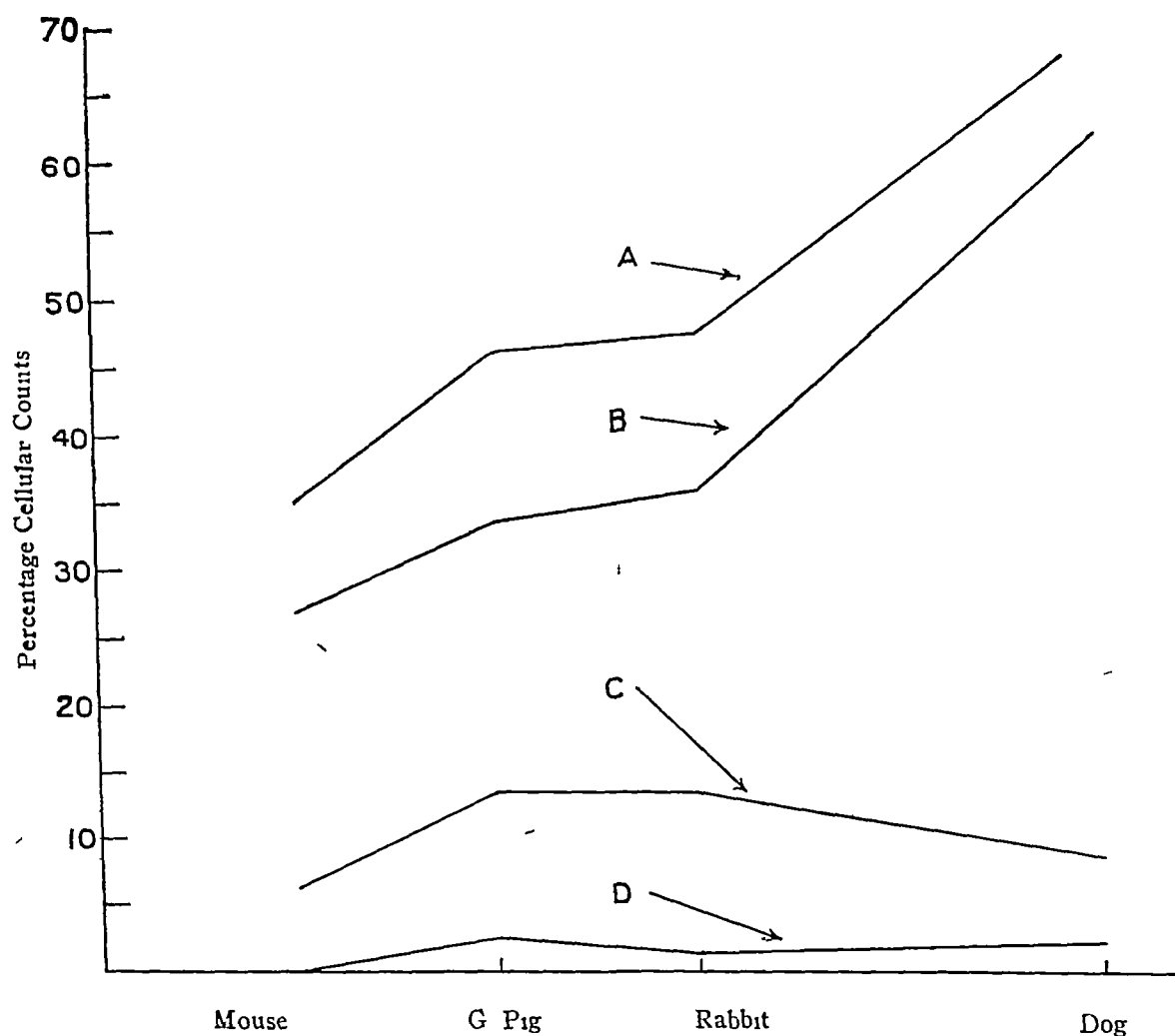


Fig 2—Showing the relation of polymorphs and oxyphils in blood to their respective forms of myelocytes in the bone marrow

- A Polymorphs + neutrophilic transitional myelocytes in marrow
- B Polymorphs in blood
- C Oxyphil myelocytes in marrow
- D Oxyphil leucocytes in blood

stage of the mouse, guinea-pig and rabbit are now in the dog producing transitional myelocytes to a greater extent Thus it is evident that bone marrow too, in higher forms, takes up a selective leucopoietic function in response to the greater needs of the organism, in that the marrow shows increased production of transi-

concerned In most cases a definite increase of reticular normocytes has been noted towards the end of the period of iron administration The above fact coupled with an indication of increased maturation of megaloblasts heralds an increased activity of the red blood-cell forming organs like spleen and bone-marrow as results of iron treatment

Most of the reticulocytes appear bigger in size than the ordinary red blood-cells Reticulocytes maintain their shape better and seem to have less tendency to run into rouleaux than the ordinary erythrocytes examined under the described conditions

The spleen sections of both the control and experimental puppies give a very faint microchemical reaction of ionizable iron specially in the venous sinuses

Samples of tissues from dogs have been analyzed for their iron content

The degree of iron contents of spleen, liver and kidney in normal dogs per gramme weight is in the order described The administration of ferri et ammon citras (green) in normal puppies in moderate or in heavy doses both orally or intravenously has hardly any effect on the final iron content of the blood after an experimental period of 15 days though a temporary increased output of reticulocytes has been noted A huge storage of iron is noticed in spleen, liver and kidney which varies directly with the increase of dosage and time Intravenous administration of the drug specially favours the storage in the aforesaid organs The nature of the iron that is stored under these circumstances is mostly that of a protein compound of iron and is not of the nature of any inorganic increase inasmuch as no readily ionizable iron can be detected

In conclusion, I wish to express my sincere thanks to Professor E W H Cruickshank for suggesting the problem and for his helpful criticisms during the course of these experiments I am also indebted to Dr B Narayana for assistance in certain of these experiments

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total percentage of transitional myelocytes and polymorphs in the marrow increases steadily in the same order (Fig 2)

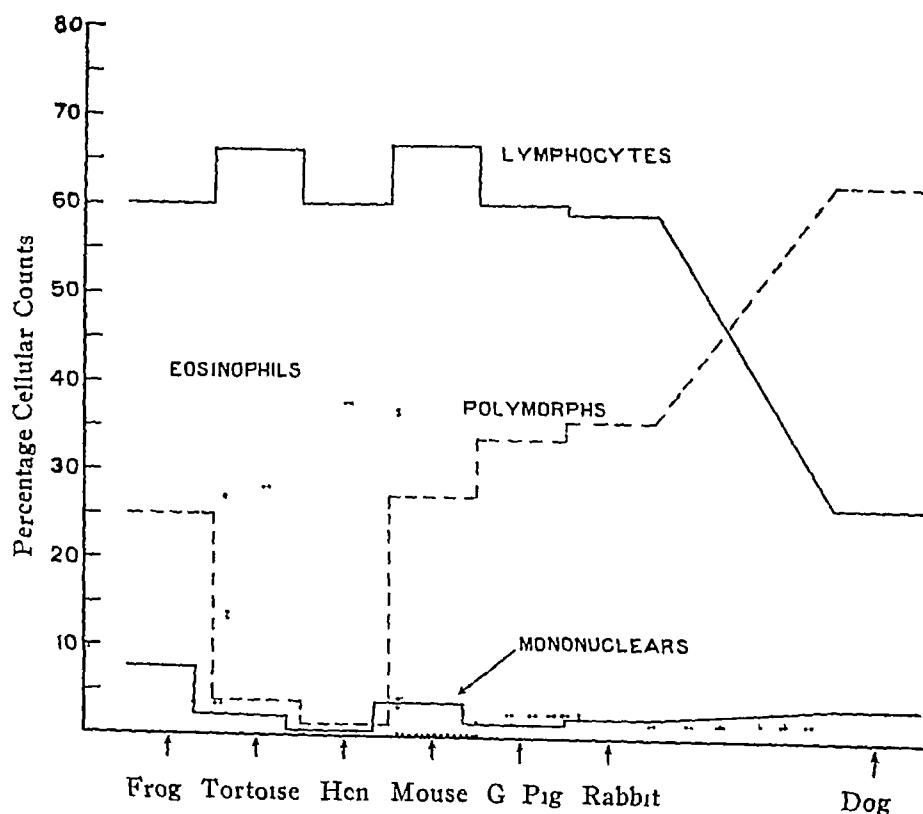


Fig 4—Showing the relation in the blood of various vertebrates of polymorphs, lymphocytes, mononuclears and eosinophils

The above observations undoubtedly lend support to the view that the chief origin of the polymorphs is in the red marrow and it can clearly be stated at this stage that these transitional myelocytes in their later life are the immediate precursors of a fairly large number of polymorphs in the circulating blood of the lower vertebrates

Secondly, polymorphs are in part the manufactured products of the spleen. In support of this statement the following evidence may be considered —

(1) In the spleen sections of puppies, polymorphs were found in the various stages of formation. The nuclei of these appeared quite coarse, convoluted and sometimes multilobulated as if they were in the prophase stage of nuclear division. Some of them were also found in advanced stages of mitosis.

(2) In the case of the infected puppies of later experiments, the differential count in spleen smear preparations showed a marked increase of polymorphs. In sections of the spleen numerous polymorphs were seen in the venous sinuses of dogs suffering from localized streptococcal and staphylococcal infection.

has been relied upon as evidence of cure, in others half the patients were partially treated, and of the rest in no instance, that the writer has been able to discover, has the whole series of results, including cures, deaths and relapses, been reported

*Dr Foster's cases*—Dr Percy Foster (1924) published a report on a small series of cases, he was only able to check the immediate result by spleen or liver puncture without culture of the material, but, as the patients were coolies on one of the estates of which he was medical officer, he was able to keep in touch with them for a considerable time. He very kindly sent me the whole of the case sheets of the patients who had been treated by urea-stibamine. Notes had been most carefully kept so that I was able to get practically all the information that was required from these case sheets. The results are summarized in an appendix to this paper.

*The brands used*—Three brands of urea-stibamine have been used in these experiments

*A*—Urea-stibamine, which Dr Brahmachari very kindly gave to me free for trial

*B*—Stiburea, prepared by the Union Drug Company, Calcutta, also given free for purposes of trial. [This was the first occasion on which any brand, other than the original urea-stibamine (Brahmachari), had been tested clinically and it was on our advice that this preparation was placed on the market.]

*C*—A sample prepared by Mr N R Chatterjee of the Chemical Department of the Calcutta School of Tropical Medicine

It has been pointed out elsewhere (Ghosh, Chopra and Chatterjee, 1928) that different batches of urea-stibamine have different percentages of antimony, varying between 44 and 20 per cent. More than one batch was used in carrying out these treatments, but none of them contained less than 30 per cent of antimony.

*Relative toxicity*—The relative toxicity of this drug is low, although not as low as that of some of the other pentavalent compounds of antimony. The toxicity of each brand was tested separately and in the case of brands *A* and *B* both the English and the Japanese varieties of white mouse were used. The toxicity of *C* was not tested accurately, but it was not appreciably higher than that of the other two. The injections were given intravenously into the tail vein. The tables below give the toxicity of brands *A* and *B*.

*The patients*—The patients were all in-patients in the Carmichael Hospital for Tropical Diseases, 6 were Anglo-Indians and 64 Indians.

The patients were not in any way selected. Consecutively admitted patients were placed on treatment. A few patients were *in extremis* when admitted and were, therefore, not put on to any form of treatment, and in a few other instances the specific treatment was withheld for a time until the condition of the patient improved sufficiently to stand the antimony injections. Those treated by brand *A* were in hospital from July to December 1925, and those treated with brand *B* between January and December 1926. Except for 5 cases treated in 1923 with

already been pointed out, this corroborates the statement that the polymorphs of the blood take their origin from the neutrophilic transitional myelocytes of bone marrow. The few transitional leucocytes that appear in blood do not seem to have any special function, but they come from the marrow in a regulated flow, and the percentage in blood corresponds to the percentage of transitional myelocytes of the marrow. Transitional cells in blood keep some resemblance in nuclear appearance with the transitional myelocytes in marrow. While in the spleen these transitionals do not occur to any great extent, one is not justified in ascribing any function to the spleen in explanation of their appearance in the blood. In fact the percentage of transitionals in the spleen is dependent upon their percentage in the blood.

Under the general classification of leucocytes in the blood, transitional forms are grouped with monocytes, but this grouping rests on the similarity of their non-granular cytoplasm and on a slight resemblance in nuclear shape, and not on any evidence of their origin from any common parent. In a word the transitional leucocyte is here regarded not as an intermediate form between a large mononuclear and the monocytic form from which it arose, but as a cell derived wholly from the transitional myelocyte of the bone marrow. We are convinced from the study of the bone marrow that so far as the origin of polymorphs from marrow is concerned, they take origin from the leucoblasts through the intermediate stage of transitional myelocytes. Again a few of these myelocytes undergoing a little change represent the transitional forms of blood, whereas mononuclears come mostly from the venous sinuses and pulp of the spleen. If they have their origin in the bone marrow, they must arise directly from leucoblasts or directly from endothelial leucocytes of the venules in the bone marrow.

*On Oxyphilic Leucocytes of Blood*—Blood of the frog, tortoise and hen are remarkably rich in their eosinophilic content as compared to the mammals, e.g., mouse, guinea-pig, rabbit and dog. Side by side with this a greater percentage of oxyphil myelocytes has also been observed in marrow films of these lower animals (Figs 1 and 2).

The same fact has been observed from another standpoint. Infection of *ankylostoma duodenale* in dogs of some of our experiments resulted in a high percentage of eosinophils in the blood. On examination of the bone marrow, it was found that there was a concurrent increase of oxyphil myelocytes. But the spleen did not show any increased activity in this direction.

From the above data naturally it follows that oxyphil myelocytes of bone marrow give rise to the oxyphil cells of blood as their number in blood corresponds to that of oxyphil myelocytes in marrow. In fact, the percentage of oxyphil myelocytes in bone marrow has always been found to be conspicuously higher than the percentage of the oxyphil leucocytes in the blood.

#### SUMMARY AND CONCLUSIONS

In the frog, the spleen is the sole organ for erythropoiesis because bone marrow in this species is exceedingly poor in erythroblastic elements. The

history that the patient has remained in perfect health for six months after discharge has been accepted as the final criterion in this series. In every instance, however, a spleen or liver puncture with culture was done before the patient was discharged from the hospital and, as the time factor appears to be an important one, an interval of at least 10 days was allowed to elapse after the last injection before the puncture was carried out in all but the first few cases of this series.

Of the patients who were eventually completely cured, the spleen puncture culture was negative in 37 and positive in 2 at the time of discharge, in the two latter instances no further clinical signs or symptoms of kala-azar were noted.

*Dosage*—No very uniform system of dosage was adopted. For an adult an initial dose of 0.1 gramme was given followed by a second dose of 0.2 gramme, and third and subsequent doses of 0.25 gramme, children were given proportionately smaller doses, but never less than 0.05 gramme as an initial and 0.1 gramme as a maximum dose. The injections were given intravenously three times a week. In most instances 10 or 11 injections were given, but in a few cases, in which the reaction to treatment was slow, and in resistant cases, more injections were given.

*Results of treatment*—The immediate results of treatment were as follows—

Series	A	B	C	Total
Discharged as cured	20	43	1	64
Died	1	3		4
Failures	2			2
TOTALS	23	46	1	70

*The failures*—Both these were Indian youths who were definitely resistant to all forms of treatment. One had previously received a course of injections of, first, No. 693 and, later, Antimosan without showing any improvement, this boy was given 2.1 grammes of urea-stibamine in 11 injections amounting to a total relative dose of 3.86 grammes. The other had been treated by sodium antimony tartrate followed by Antimosan, a full course in each instance, he was then given 3.3 grammes of urea-stibamine in 17 injections, amounting to a relative total dose of 5.5 grammes. In neither case was there any improvement during or after the course of injections, both boys are reported to have died subsequently.

*The deaths*—One patient, a child, died with hyperpyrexia and hæmatemesis very shortly after the second injection, another died very suddenly three days after the fifth injection, a third died 24 hours after the second injection, and the fourth died of hyperpyrexia 4 days after the third injection.

# THE EFFECT OF IRON ON THE HÆMOGENIC ORGANS

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To study the effect of iron salt on the hæmogenic organs of the puppies, estimations of the iron content of blood, blood plasma, spleen, liver and kidney from different individuals were made with the idea of arriving at some approximate standard of iron content in these tissues. Variations due to differences in age, body weight and apparent condition of the dogs are naturally found, but the variations are not of a large order.

In the investigation of this problem estimations of iron in the ferric form have been carried out before, during and after the administration of ferri et ammon citras. The drug was given orally as well as intravenously to puppies selected in order to have them approximately of the same age and weight. They were fed on a mixed diet of meat and rice. A control series was always kept throughout the experiments. The work was subdivided as follows —

*Work on the animal before and during the experimental period*

- (1) Differential blood count
- (2) Reticulocyte count
- (3) Estimation of blood iron

*Work done on the animal at the end of the experimental period*

- (1) Spleen section, spleen smear and bone-marrow picture, differential, reticulocyte count
- (2) Micro-chemical detection of inorganic iron in spleen section and bone-marrow
- (3) Estimations of iron content of blood, spleen, kidney and liver

## HISTOLOGICAL OBSERVATIONS

The blood picture has thrown no light on the increased or decreased formation of leucocytes in puppies during the period of administration of ferri et

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\* Working under a grant from the Indian Research Fund Association



*The leucocyte count on discharge*—The white blood count was recorded in 56 of the cases, in 48 of these it was above 6 000 per c mm, in 6 between 5,000 and 6,000, and in 2 below 5,000. The mean count of the whole series was 7,851, of series *A* 8,890 and of series *B* and *C* 7,340.

*Comparison of series A and B with reference to progress under treatment*—There appears to be very little to choose between these two series, the fall of temperature, the reduction in the size of the spleen and the increase in weight appear to be very slightly better in series *B*, but on the other hand the blood counts in series *A* are very distinctly better. The results are quite in keeping with the claim that the two preparations have the same chemical composition. Subsequently in this paper the results are not analyzed separately.

#### DOSAGE IN CASES IN WHICH A COMPLETE CURE WAS EFFECTED

Of the 49 patients who were completely cured 4 had previously received treatment and had relapsed, and 45 were being treated for the first time.

*Number of injections*—The number of injections given in these 49 cases is recorded below—

Number of injections	Number of previously untreated cases in which given	Number of resistant cases in which given
6	1	
8	2	
9	1	
10	14	
11	16	
12	2	1
13	4	
14	2	
15	2	1
16	1	
20		1
21		1
TOTALS	45	4

The mean of the whole series of 49 patients is 11.53. The mean number of injections given in the 45 ordinary cases was 11.04 and in the resistant cases 17.0 injections. The patient who relapsed received 12 injections.

TABLE II

*Differential Counts of Blood, Bone marrow and Spleen Smear of the Puppies under Experimental Conditions*

	Method of iron administration		Blood Average percentage	Bone marrow Average percentage	Spleen smear Average percentage.
Puppy No 7	Oral	Polymorphs	64.5	14	20
		Lymphocytes	27	13	66
				Leucoblasts 12	Splenocytes 3
		Mononuclears	2	5	4
		Transitionals	2.5	Myelocytes {	2.5
		Eosinophils	4		4
		Basophils	nil	nil	0.5
Puppy No 8	Intravenous	Polymorphs	74	8.5	38
		Lymphocytes	18.5	15	52
				Leucoblasts 14.5	Splenocytes 2
		Mononuclears	3	6	2
		Transitionals	4	Myelocytes {	3
		Eosinophils	0.5		3
		Basophils	nil	0.5	nil
Puppy No 3	Intravenous	Polymorphs	57	Not recorded	12
		Lymphocytes	33		63
					Splenocytes 7
		Mononuclears	3		8
		Transitionals	6		5
		Eosinophils	1		5
		Basophils	nil		nil
Puppy No 6	Oral	Polymorphs	59	8	16
		Lymphocytes	29	23	72
				Leucoblasts 17	Splenocytes 2
		Mononuclears	3	3	3
		Transitionals	3.5	Myelocytes {	2.5
		Eosinophils	5.5		4
		Basophils	nil		0.5

The mean of the relative total dose given to the 49 patients is 3.12 grammes, to the 45 previously untreated ones 3.00, and to the 4 resistant patients 4.54 grammes. The patient who relapsed received a total relative dose of 3.5 grammes.

*The dosage according to age*—The mean actual and relative doses according to the ages of the patients are shown below—

Age group	Number of patients	Mean actual dose, grammes	Mean relative dose, grammes
Under 10 years	6	1.37	4.09
10 years and above, but less than 20 years	10	2.35	3.70
20 years, or above	33	2.42	2.78

The patient who relapsed was an adult, so that the relapse rate amongst 34 adults receiving a relative dose of 2.80 grammes was about 3 per cent. Again in this series the younger patients received relatively larger doses.

*The cure rate*—The usual difficulty arises in estimating the cure rate on account of the fact that only one patient relapsed. In this series the patient who relapsed received more than the mean of the number of injections of the whole series, and more than the mean of the total actual and relative doses, respectively. We can only say that of the previously untreated patients whose subsequent history we know, of those who received 12 injections, or less, only one out of 37 relapsed, that of those who received a total dose of 3.00 grammes, or less, one out of 45 relapsed, and that of those who received a total relative dose of 4.0 grammes, or less, one out of 42 relapsed. So that with these dosages the cure rate amongst those who survived the full course was over 97 per cent.

*Complications and sequelæ*—In four instances jaundice occurred within 2 months of discharge (1 case of series *A* and 3 of series *B*).

One patient (series *A*) had severe hepatitis so that treatment had to be discontinued temporarily.

One patient remained in good health for 2 years, but at the end of this period he was re-admitted with huge enlargement of the spleen, general anasarca and a history of fever for two months. No parasites were found in the spleen puncture material and the culture was 'negative'. The patient eventually died of œdema of the lung.

One patient developed dermal leishmaniasis a year after discharge.

#### SUMMARY AND CONCLUSIONS

Urea-stibamine is a drug of very considerable value in the treatment of kala-azar. It is a drug with a low relative toxicity in mice and is well tolerated by man in comparatively large doses.

oral administration causes a temporary increase of reticulocytes in blood specially towards the end of the experimental period

*Method for staining Reticulocytes*—The method that we adopted was but a slight modification of the one that is advocated for staining these cells in freshly drawn blood. Reticulocytes cannot be stained in fixed films by any ordinary method

A 0.3 per cent solution of brilliant cresyl blue is prepared in absolute alcohol and kept as a stock stain. A very clean slide is taken and an even film of the above stain is drawn on the slide in the usual way. The film dries immediately. The filmed surface of the slide is very gently rubbed twice or thrice with a dry smooth paper. Then a minute fraction of a drop of blood is taken on a coverslip and the coverslip is immediately pressed hard on the stained slide. The reticulocytes are stained within a few minutes. The slides are then examined under the oil immersion lens. The red blood-cells including reticulocytes are counted in thousands in the portions of the field where counting is conveniently possible. In addition to hæmoglobin in the stroma the reticulocytes are made up of reticulum and of granules, the amount of reticulum depending upon the age of the cell. It is the reticulum and granules which are stained blue black. Another interesting feature with these reticulocytes is that they maintain their shape better and have less tendency to run into rouleaux than the ordinary erythrocytes examined under the aforesaid conditions.

*Microchemical Tests of Inorganic Iron in Spleen Sections and Marrow Film*—Spleen sections of control puppies as well as of those treated with iron salt for more than two weeks show equal indifference to the reaction of inorganic iron by the Prussian blue method and to the hæmatoxylin test. In the Prussian blue method, sections obtained from tissues fixed in alcohol are immersed in a mixture of an equal volume of 0.5 per cent iron-free hydrochloric acid and 1.5 per cent potassium ferrocyanide for five to ten minutes. In case of the presence of the ferric salt, sections take up either a diffuse blue stain or a deep blue stain at certain spots. When the ferrous iron is present, it gives the reaction with ferricyanide. Again, when the above reactions are positive, after having kept the slide for some time over a hot copper plate, the test should not be regarded as indicating the presence of simple inorganic iron, because in the latter case it may be argued that some iron may have its origin from the splitting of hæmoglobin derivatives or the degeneration products of hæmoglobin.

In the hæmatoxylin method sections are kept for about eight to ten minutes in 0.5 per cent solution of absolutely pure hæmatoxylin in redistilled water and afterwards excess of stain is removed by subjecting the preparation to alcohol and ether treatment. The portions containing inorganic iron are stained blue black or blue violet.

The above tests show the faintest reaction of inorganic iron in the spleen sections of puppies. The same fact is also true for puppies previously treated with iron salt. Therefore it may be said that a slight trace of inorganic iron in the spleen of both normal and experimental puppies is microchemically detectable.

	ACTUAL DOSES.			RELATIVE DOSES		
	Ordinary	Resistant	Relapses	Ordinary	Resistant	Relapses
Less than 1 gramme	2		2			1
1.0 to 1.49 gramme	19	1		4		1
1.5 to 1.99 „	10			6		..
2.0 to 2.49 grammes	25	6		18		..
2.5 to 2.99 „	8			15		
3.0 to 3.49 „	9	1		9	2	.
3.5 to 3.99 „				4		
4.0 to 4.49 „				5	1	
4.5 to 4.99 „				3		
Over 5 „				9	5	
TOTALS	73	8	2	73	8	2

	<i>Actual dose</i>	<i>Relative dose</i>
Mean dose of whole series including the relapses	2.01 grammes	3.40 grammes
Mean dose in cured cases only	2.05 „	3.46 „
Mean dose in previously untreated cases	2.03 „	3.24 „
Mean dose in 'resistant' cases	2.23 „	5.45 „

The reaction of inorganic iron in marrow films has always been negative

#### ESTIMATION OF IRON IN BLOOD AND DRIED SPLEEN

Estimations of iron in several samples of fresh blood, dried spleen, liver and kidney taken from different dogs were carried out according to a recent colorimetric method described by F S Fowweather. The only step we added to the method is that whenever we required a tissue for iron estimation, we killed the animal by bleeding it thoroughly from the carotid artery. The method is fully noted below.

*Method of Estimation of Iron in Blood (Fowweather)*—One c c blood is measured into a test tube containing 4 c c of distilled water. After thorough mixing, 1 c c of this diluted blood is transferred to a Pyrex test tube (200 × 25 mm) followed by 1 c c of concentrated sulphuric acid. The tube is clamped and held at an angle of 40° to the horizontal. The contents are boiled rather vigorously until practically all the water has been driven off and white fumes begin to be evolved. Heating is discontinued for about half a minute, after which time 0.5 c c of 'perhydrol,' i.e., 100 vols hydrogen peroxide is added to the tube, drop by drop, from a test pipette. Boiling is then repeated. A brisk evolution of oxygen occurs and the solution in the tube assumes an amber colour. When white fumes are again evolved, heating is again discontinued and after cooling for half to one minute a further 0.5 c c of 'perhydrol' is added as before. Heating is resumed and the solution usually becomes colourless. If all colour has not disappeared, a further 0.3 c c of 'perhydrol' is added. Heating is continued for one minute after the solution has become colourless. The solution is now allowed to cool completely, then it is diluted with about 5 c c of distilled water and transferred to a 50 c c stoppered graduated flask. Into another similar flask are placed 1 c c of a standard iron solution containing 0.1 mg iron per c c and 1 c c concentrated sulphuric acid. Water is added to both flasks up to a volume of about 18 c c after which 25 c c acetone are added. The contents of the flasks are thoroughly mixed and allowed to cool to room temperature. To each flask are then added 5 c c of 3 M ammonium thiocyanate, the contents mixed and made up to the mark. The two solutions are then compared in a colorimeter.

If the standard is set at 20 mm then

20

— × 50 = mg iron per 100 c c blood,

R

where R is the colorimeter reading of the solution tested

*Method of Estimating Iron in Spleen*—The organ or tissue to be examined is minced and washed with water to remove blood. It is then dried in a steam oven. The dried material is then ground in a mortar until it passes through fine muslin.

One gramme of this material is weighed into a 300 c c Kjeldahl flask to which are added 10 c c concentrated  $H_2SO_4$ . The flask is heated in a fume

and in some instances less than 4 per cent of the mice subjected to this dosage succumbed. In order to make sure that the drug had not increased in toxicity during its transport to India, the toxicity was tested by the writer, the English variety of mouse was used, average weight 25 grammes, and the injections were given into the tail vein. The details are given in the table below —

Dose in grammes per kilo-gramme body-weight of mouse	Number of mice	Number surviving
0.300	5	5
0.350	5	4
0.400	5	4
0.500	5	1
0.600	5	0

It is obvious that there was no serious change in the drug by the time it reached India, although these observations do suggest that there was some slight increase in the toxicity, or that the mice used by us were more susceptible.

*The patients*—The patients were all in-patients in the Carmichael Hospital for Tropical Diseases, 14 were Anglo-Indians, 7 of each sex, 42 were Indians, of whom 8 were females, and 1 was a male European.

They were not in any way selected. Consecutively admitted patients were placed on treatment during the times that the drug was available, the first 10 patients were treated between October and December, 1924, and the remainder between April and December, 1926. The patients were either previously untreated, or had received a definite course of injections and had relapsed, in the latter case they are classed as 'resistant' cases.

*The diagnosis and the proof of cure*—Only cases in which the parasites were actually demonstrated are included in this series and the criteria of cure was the same as that previously adopted by the writer (1926). In most instances a spleen or liver puncture with culture was performed before discharge, in 8 instances the culture showed the presence of parasites, in the two totally resistant cases, in three other cases in which a relapse occurred, in two cases in which a complete cure was effected, and in one patient who was lost sight of. Of the remaining six relapse cases the culture was 'negative' in 3, it became contaminated in two others, and in one case it was not done.

*The dosage*—No very rigid system of dosage was adopted. About two-thirds of the previously untreated patients received 10 injections, but occasionally when the individual doses had to be modified on account of vomiting, it was thought advisable to give a few more injections, and in resistant cases 12 or more injections were given. The initial dose was usually 0.1 gramme and the maximum dose 0.3 gramme in those cases in which the drug was well tolerated.

TABLE V

*Some Results of Iron Analysis in Various Tissues of Puppies In case of Spleen, Liver and Kidney, results have always been expressed in milligrammes per 100 grammes of Dried Tissue and in case of Blood or Plasma per 100 ccs*

## Control

Puppy No		Spleen	Liver	Kidney	INITIAL		FINAL	
					Blood	Plasma	Blood	Plasma
5	Normal, killed at the beginning Do	(i) 71.4 (ii) 72.4	(i) 53.3 (ii) 54.1	(i) 54.0 (ii) 54.5	(i) 40.0 (ii) 39.2	(i) 4.0 (ii) 3.8		
4	Normal, killed at the beginning Do	(i) 74.2 (ii) 73.6	(i) 49.4 (ii) 50.2	(i) 43.0 (ii) 44.3	(i) 39.1 (ii) 40.8	(i) 3.4 (ii) 4.1		
2	(Control) After 2 weeks' period of the experiment Do	(i) 76.1 (ii) 78.0	(i) 44.1 (ii) 43.4	(i) 37.3 (ii) 38.6	(i) 40.0 (ii) 41.3		(i) 40.0 (ii) 39.3	(i) 12.5* (ii) 11.7
1	(Control) ( Do )	(i) 93.8 (ii) 94.2	(i) 51.2 (ii) 52.1	(i) 44.4 (ii) 43.8	(i) 39.3 (ii) 40.8		(i) 37.9 (ii) 38.5	(i) 5.1 (ii) 4.8
9	(Control) ( Do )				(i) 34.8 (ii) 35.2		(i) 32.9 (ii) 33.4	
15	(Control) ( Do )				(i) 43.5 (ii) 44.1		(i) 46.2 (ii) 45.7	
14	(Control) ( Do )				(i) 44.3 (ii) 43.5		(i) 43.7 (ii) 42.9	

\* Partial laking took place, hence the plasma was mixed with hæmoglobin and the result was high



## Splenic Enlargement

	All	Resistant cases	Relapsing cases
Average size of spleen before treatment in inches below the costal margin	3.2	5.2	2.9
After treatment—			
Number in which the spleen was not palpable	18		3
Just palpable	21	3	2
2" below costal margin	5		1
2½" " " "	2	1	2
3" " " "	4		
4" " " "	2	1	1
Size not noted	1		
TOTALS	53	5	9

*Weight*—Three patients lost weight, the average loss was 3.50 lbs. In three others there was no change in weight, and 46 gained in weight, the average gain being 7.95 lbs.

Of the patients that were discharged as cured, excluding those that eventually relapsed, two lost weight, in two the weight was unaltered, and 39 gained in weight (mean 8.00 lbs). (In one instance the weight on discharge was not recorded.) Of the 43 patients that were eventually cured the net gain had a mean of 7.20 lbs.

Of those that relapsed 7 gained in weight (mean 7.7 lbs), one lost (7 lbs), and one remained the same weight, of these 9 patients the mean of the change in weight was a gain of 5.21 lbs.

The mean of the gain in weight in the resistant cases was 8.8 lbs.

Of the whole series the mean gain in weight was 6.85 lbs.

*The leucocyte count*—In 9 instances the count was not recorded. In only two instances it was below 5,000 per c mm and in 6 it was below 6,000. In the remaining 37 cases it was 6,000 or more, the mean being 7,061. In 8 of the 9 cases in which there was a relapse the mean of the count was 7,320, in other case it was not recorded.

In the 5 resistant cases the mean was 6,802.

#### DOSAGE IN THE CASES IN WHICH A COMPLETE CURE WAS EFFECTED AND IN THOSE CASES IN WHICH A RELAPSE IS KNOWN TO HAVE OCCURRED

Of the 35 patients that were eventually cured 33 had previously had no treatment and 2 were resistant cases, and of the 9 cases in which a relapse occurred 6 had previously had no treatment and 3 were resistant cases.

TABLE V—*concd*

Puppy No		Spleen	Liver	Kidney	INITIAL		FINAL	
					Blood	Plasma	Blood	Plasma
10	Received orally ferri ammon. citras 0.125 gramme daily with egg for 15 days				(1) 41.7		(1) 40.9	
	Do				(11) 41.5		(11) 41.8	
13	Received orally ferri ammon. citras 0.125 gramme daily with egg for 15 days				(1) 43.1		(1) 44.4	
	Do				(11) 42.8		(11) 43.8	
6	Received orally ferri ammon. citras 0.25 gramme daily with egg for 3 weeks	(1) 100.5	(1) 114.6	(1) 66.7	(1) 39.8		(1) 36.7	(1) 5.2
	Do	(11) 101.2	(11) 115.8	(11) 67.4	(11) 41.6		(11) 37.2	(11) 4.6
7	Received orally 0.25 gramme of ferri ammon citras daily with raw egg for 15 days	(1) 140.3	(1) 72.7	(1) 74.1	(1) 41.5		(1) 43.2	(1) 4.2
	Do	(11) 138.6	(11) 73.4	(11) 74.8	(11) 42.3		(11) 43.6	(11) 4.8

The mean of the total dose given to the 35 cured patients was 2.14 grammes, to the 33 previously untreated patients 2.16 grammes and to the resistant ones 1.75 gramme. The mean dose of the 9 relapsing patients was 3.45, of the 6 previously untreated patients 2.36 and of the resistant ones 2.62 grammes.

*The relative dose* — The patients can be grouped according to the relative dose (grammes per 100 lbs body-weight) administered, as follows —

Group	PREVIOUSLY UNTREATED PATIENTS		RESISTANT CASES	
	Cured	Relapsed	Cured	Relapsed.
1.5 to 1.99 gramme	3	2	1	
2.0 to 2.49 grammes	5	2		
2.5 to 2.99 „	10	1		
3.0 to 3.49 „	7	1		2
3.5 to 3.99 „	1		1	
4 grammes or over	7			1
TOTALS	33	6	2	3

The mean of the total relative dose given to the 35 cured patients was 3.17, to the 33 previously untreated patients 3.18 grammes and to the 2 resistant ones 2.89 grammes. The mean of the total relative dose given to the 9 relapsing patients was 2.80 grammes, to the 6 previously untreated patients 2.42 grammes and to the 3 resistant ones 3.56 grammes.

*Dosage according to age* — The relationship of the means of the actual and relative total doses in the various age groups is shown below —

Age group	Number of patients	Number relapsing	Mean actual dose in grammes	Mean relative dose in grammes
Under 10 years	8		1.34	4.13
10 but under 20 years	18	4	2.45	3.25
20 years or over	18	5	2.33	2.48

The tendency was to give the younger patients relatively larger total doses. The relapse rate is highest amongst the adult groups who received the smaller relative doses.

though no reason can be assigned to such a different nature of storage in these organs

On looking at the tabulated results of iron content in various tissues, it appears that the major portion of the iron that is stored during a similar period of time is greater by intravenous administration than by oral feeding, and naturally it should be, as some portion might be unabsorbed and lost by way of the alimentary canal when the drug is given orally

There seems to be a very high limit for storing iron in tissues in case of intravenous administration as it can be noticed that with the increase of dosage there is a direct increase of storage. But in case of oral administration it seems that the limit is not easily reached, for example, puppy No 6 received iron salt orally, for a little over three weeks, 0.25 gramme a day, its store of iron in liver, spleen and kidney was 282 milligrammes per 100 grammes of each of these dried tissues. Whereas puppy No 8 received 0.25 gramme for nearly fifteen days intravenously, it stored 441 milligrammes, being much in excess to that of puppy No 6

#### QUALITATIVE TEST OF IRON IN THE SPLEEN

To investigate the nature of iron that is stored up in the spleen after the administration of ferri et ammon citras, the animals were first allowed to pass 36 to 48 hours' rest after having a prolonged treatment of the drug. Then they were bled to death, and the spleen was removed, minced and dried thoroughly in a steam oven. The dried tissue was finely powdered in a clean glass mortar.

Of the powdered sample, 0.5 gramme was treated with boiling water for 5 to 10 minutes. The whole mixture was filtered, the residue was kept apart for subsequent treatment and the filtrate was boiled again for about 2 minutes and refiltered. The fairly clear filtrate thus obtained was acidified with dilute HCl acid (iron free) and all the usual tests for ferrous and ferric salts were applied. These tests always pointed to the absence of any water soluble iron salt, including ferri et ammon citras, in the organ. Further, the residue on the filter paper of the previous operation after being repeatedly washed with hot redistilled water was transferred to a big test tube and treated with 2 per cent hydrochloric acid. The whole mixture was filtered after a few minutes and the presence of iron salt was sought for in the filtrate. This acid (dilute) extract of the organ also showed the absence of ionizable iron salt including the phosphate. The fact that microchemically only very minute traces of inorganic iron can be detected in the sections of spleen of both the control and the experimental puppies shows that the iron in the tissue is not free but is in some colloidal form. In all probability this increase of iron is in the form of a colloidal combination of protein and iron, like 'ferratin,' as was observed by Sehmedeberg in the liver some years ago.

#### SUMMARY AND CONCLUSIONS

The administration of moderate doses of ferri et ammon citras (green) in normal puppies does not alter the blood picture as far as the leucocytes are

of them a considerable amount, too much importance cannot be attached to this point, but on the whole the gain was less amongst those that relapsed

*Splenic enlargement*—The mean size of the spleen before treatment amongst the patients who eventually relapsed was less than the mean of the whole series. This suggests that amongst those relapsing the duration of the disease prior to treatment was shorter than amongst the rest. A reference to the notes shows that in three of the relapsing patients the disease had lasted less than a month, whereas amongst the rest of the patients in only two cases was it of so short a duration.

In four out of nine relapsing patients the spleen was measurably enlarged on discharge, this was the case in a much smaller proportion of the remainder. So that reduction in the size of the spleen may be looked upon as an indication that the patient is cured, but too much importance must not be attached to this sign.

*The blood count*—Little information can be gained from this observation as the mean of the white blood count amongst the patients that relapsed was greater than amongst the remainder.

*Presence of parasites on discharge*—A 'positive' liver culture was obtained in two cases in which a complete cure occurred without further treatment, and in 3 of the relapsing cases the culture was 'negative'. Thus, this test cannot be considered as the final criterion of cure (as has been previously observed), but it gives an indication of some prognostic value.

#### SUMMARY AND CONCLUSIONS

Stibamine glucoside is a drug of considerable value in the treatment of kala-azar. It is a drug of very low toxicity in mice and is well tolerated by man in comparatively large doses.

The drug can be used with safety in the treatment of kala-azar in an unselected series of 57 patients only two died during treatment.

In this series 53 patients were discharged as cured, of these the subsequent history of 44 is known, 35 being cured and 9 relapsing.

Of the patients whose subsequent history is known, 39 had previously received no treatment, of this group the mean actual total dose was 2.19 grammes, the relative (per 100 lbs. body-weight) total dose 3.06 grammes, and the relapse rate 15.4 per cent. Of 16 patients who received a relative dose of more than 3 grammes (mean 4.32 grammes), 1 (or 6 per cent) relapsed.

Little prognostic information can be obtained from observation of the progress of the patient under treatment, the rate of the reduction in the size of the spleen appears to give the most reliable indications.

#### ACKNOWLEDGMENTS

My thanks are due to Lieut-Col C. M. Wenyon, C.M.G., C.B.E., F.R.S., Director of the Wellcome Research Bureau and to Dr. Henry of the Wellcome Chemical Research Laboratories for a generous supply of stibamine glucoside for the purpose of this trial.

# THE PENTAVALENT COMPOUNDS OF ANTIMONY IN THE TREATMENT OF KALA-AZAR

## Part IV.

### UREA-STIBAMINE AN ANALYSIS OF THE TREATMENT IN 70 CONSECUTIVE CASES \*

BY

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[Received for publication, October 1, 1928 ]

*The preparation*—The preparation is a combination of urea with para-amino-phenyl stibinic acid. It was first prepared by Brahmachari (1922), who gave it the name urea-stibamine. Brahmachari (1924) claimed that it was the urea salt of para-amino-phenyl stibinic acid, but subsequently modified his view. Other workers (Henry, 1925) have criticized his statements and there appears to be some doubt as to its being a true compound. Brahmachari (1922) originally gave the antimony content as 37 per cent, subsequently (1924) as 35 per cent, but samples tested at the Calcutta School of Tropical Medicine contained as much as 44 per cent.

Brahmachari (1922) was the first to use this drug clinically. He treated 8 kala-azar patients and obtained very favourable results. Samples were sent to Shortt and Sen (1923) who also reported favourably on the results of treatment in 21 consecutive cases. From this date onwards the drug has been used very extensively in India in the treatment of kala-azar. Numerous reports have been published. The preparation has yielded uniformly good results, but most of these reports have been entirely unsatisfactory. In many instances the evidence of both the disease and the cure has been clinical only, in some a blood-culture

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\* An investigation carried out under the Endowment Fund, Calcutta School of Tropical Medicine and Hygiene.

# SOME OBSERVATIONS ON THE PHARMACOLOGY OF CARDIAZOL

BY

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AND

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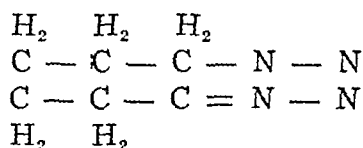
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[Received for publication, October 4, 1928]

CAMPHOR is credited with having a stimulant action on the heart, and although English pharmacologists are not enthusiastic about it, it is still in high favour with continental workers. As its action is inconstant and its administration is rendered difficult by its insolubility in water, attempts have been made to prepare for clinical use a substance, which will act better than camphor and at the same time will be soluble in water. With this end in view, cardiazol has been synthesized and put on the market by A G Knoll of Ludwigshafen-on-Rhine, Germany.

Cardiazol is a synthetic product, being a penta-methylene tetrazol, containing a bicyclic system, the same as camphor. Its chemical formula is given as follows —



It is a white crystalline powder, melting at 56° to 58°C, softening shortly before melting. It has a slightly bitter taste and is easily soluble in water and the majority of organic solvents. The solutions have a neutral reaction.

The literature supplied along with the drug is plentiful, being chiefly clinical and mostly in German. Schmidt, Hildebrandt and Krehl (1925) found that on the hearts of both cold-blooded and warm-blooded animals, cardiazol has a considerably stimulant effect, both as to amplitude and frequency of beats. These

## BRAND A

* Dose in grammes per kilogramme body-weight of mouse	JAPANESE MICE AVERAGE WEIGHT 10 GRAMMES		ENGLISH MICE AVERAGE WEIGHT 25 GRAMMES	
	Number of mice	Number surviving	Number of mice	Number surviving
0.100	5	5	5	5
0.125			5	4
0.150	5	5	5	2
0.175			5	1
0.200	5	5	5	0
0.225	5	3		
0.250	5	0		

## BRAND B

	AVERAGE WEIGHT 15 GRAMMES			
0.100	10	10	5	5
0.125	10	10	5	5
0.150	5	5	5	5
0.175			5	4
0.200			5	4
0.250			5	0

\* Whilst writing this paper I noticed that a serious error occurred in Part II of this series (Vol XV, p 181) In the table corresponding to this, the doses in grammes per kilogramme body-weight appear as whole figures, they should, of course, have appeared as decimals This mistake was repeated in Part III (Vol XVI, p 141)

urea-stibamine, this series represents the writer's whole experience with this drug up to the end of 1926

As in the previous series, the patients had either received no treatment at all, or had been given a definite course of treatment and had relapsed, the latter group are referred to as 'resistant' cases in this series

*The diagnosis*—In every case the diagnosis was made by actually demonstrating the parasite in the blood, in the spleen or liver puncture material, or by cultural methods

*Proof of cure*—As there appears to be no absolute proof of cure, the writer has adopted the system followed in his previous series of cases, a clear



convulsions are very violent and opisthotonus is maintained. One c.c. of a 10 per cent cardiazol solution, i.e., 100 mg., administered intravenously invariably produces convulsions in cats anaesthetized with urethane. Convulsions also occur in pithed animals, where the medulla has been destroyed by passing a probe through the foramen magnum. In a few experiments, only muscular twitchings were noticed. These experimental facts go to prove that cardiazol stimulates not only the medulla but also the reflex centres in the spinal cord. In this, our results confirm the findings of Blume and Schoen, quoted above.

*Peripheral nerves*—A 10 per cent solution blocks the passage of impulses in a sciatic-gastrocnemius preparation of a frog in 5 minutes. A 7.5 per cent solution produces the same effect in 10 minutes. With a 5 per cent solution, there is only a perceptible diminution of conductivity after about 15 minutes. On sensory nerves, the effect is less noticeable. A 10 per cent solution dropped into the eye of a rabbit causes some irritation which soon passes off. No local anaesthesia is produced but the corneal sensitiveness is slightly dulled after 15 minutes.

*Circulatory System*—On the isolated heart of a frog dilutions of 1 in 1,000, cause slight stimulation of the ventricular contraction. With greater concentrations, i.e., up to 1 in 200 the stimulant effect is more marked. Plate LXXII (a). Concentrations of 1 in 100 and more invariably show a preliminary depression and slowing of the heart, but this is followed by a well marked stimulation. Plate LXXII (b). Though the preliminary depression becomes more pronounced with increase of dosage, even as high a concentration as 1 in 20 does not cause any permanent damage to the heart. The preliminary depression is due to a direct action of the drug on the cardiac muscle as it still occurs after the vagus nerve endings have been paralysed by atropine. The manufacturers claim that cardiazol has great powers of restoring a heart previously damaged. Plate LXXII (c) shows that cardiazol, 1 in 100, revived the isolated heart of a frog after it was depressed by a 1 in 10,000 tartar emetic solution. Plate LXXII (d) shows revival of the heart with a 1 in 100 dilution of cardiazol after depression with chloral hydrate (1 in 250). Plate LXXII (e) shows that a heart depressed and made irregular by a 1 in 10,000 emetine solution was permanently revived by a 1 in 1,000 cardiazol solution. It will be seen that, except in the third instance, the dosage found necessary to stimulate the damaged heart is large.

On the isolated mammalian heart (kitten), 1 in 1,000 dilutions of cardiazol produce a slight but persistent stimulation of the ventricular contraction. Plate LXXII (f). The effect is more marked with bigger doses. Plate LXXII (g and h). But concentrations of 1 in 100 and more cause a preliminary depression and slowing which is followed by the characteristic stimulation. Plate LXXII (i).

The action of the drug on the intact mammalian heart was studied by means of the myocardiograph. Experiments were done on cats and dogs. The dog's heart did not respond uniformly to injections of cardiazol, but fairly constant effects were noticed on the cat's heart. Injections of 0.5 c.c. of a 10 per cent solution, i.e., 50 mg., invariably produces stimulation of both auricle and ventricle.

*Patients discharged as cured, progress under treatment*—Of the patients discharged as cured accurate information is available with reference to 51, of these one died (series *B*) in hospital following a surgical operation, one relapsed (series *A*), and 49 (16 of series *A*, 32 of series *B* and 1 of series *C*\*) received no further treatment and remained in perfect health for 6 months, or more, after discharge, that is to say, they were cured

*The number of days under treatment*—The mean number of days under treatment of the 64 patients discharged as cured was 27.84, of the 59 previously untreated patients the mean was 26.4, and of the 5 resistant ones it was 44.8 days

*The cessation of fever*—Of the 64 patients discharged as cured 5 were afebrile and 4 were febrile throughout the whole course of treatment, of one patient there is no record, and of the remaining 54 the mean number of injections given prior to the fall of temperature to normal was 4.93. Of the previously untreated patients the mean was 4.83 and of the resistant ones 6.0. Of series *A* the mean number of injections given prior to the fall of temperature was 5.53 and of series *B* 4.72

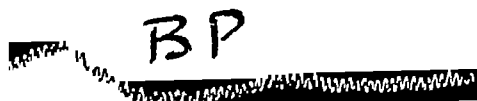
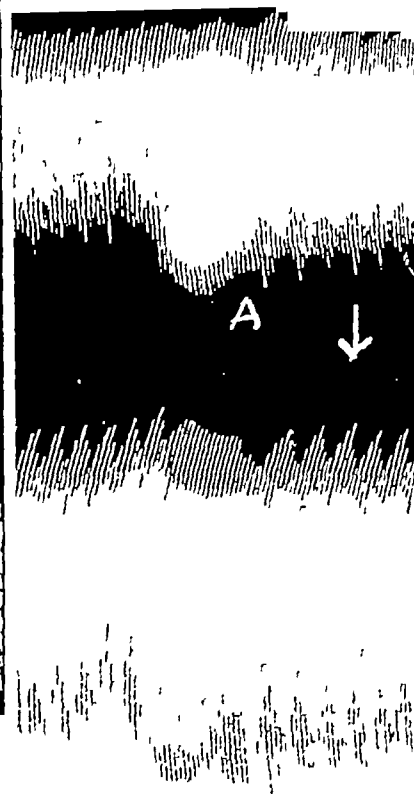
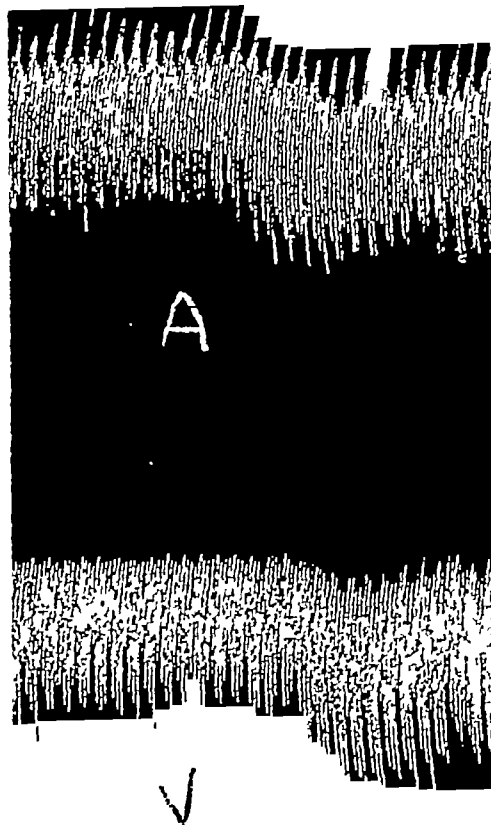
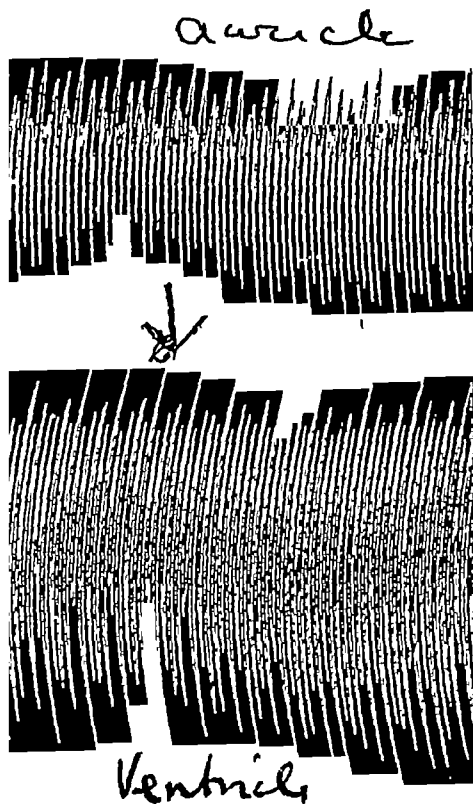
*Splenic enlargement*—The average size of the spleen of the 64 patients before treatment was commenced was 3.66 inches below the costal margin, at the time of discharge the measurements were as follows —

	Series <i>A</i>	Series <i>B</i>	Total
Not palpable	5	12	17
Just palpable	10	23	33
Palpable below costal margin, 2"	2	2	4
" " " " 2½"	1	2	3
" " " " 3"	1	2	3
" " " " 4"		2	2
Size not noted	1	1	2
TOTALS	20	44	64

*Weight*—In 3 instances the weight was not recorded. Of the remainder 52 increased in weight, 2 maintained their weight, and 7 lost weight, of the 9 patients who failed to increase in weight, 4 were of series *A* and 5 of series *B*. Of those gaining weight the average increase was 6.88 lbs, of those losing weight the average loss was 2.71 lbs. The mean net gain in weight of the whole series was 5.55, of series *A* patients 5.34 and of series *B* 5.64 lbs

\* This case is subsequently included in series *B*

PLATE LXXIII  
Myocardiogram (cat)



Cat 21 K L  
decreased

cardiazol 0.5 cc

-6 Sec

cat 3.2 K L  
urethane 14-9-31



cardiazol 0.5 cc



(a)

(b)

(c)

*The actual total dose* — The patients are divided into groups according to the actual total dose received, as follows —

Group	Ordinary cases	Resistant cases
10 to 149 gramme	6	
15 to 199 „	7	
20 to 249 grammes	15	1
25 to 299 „	16	1
30 to 349 „	1	
35 to 399 „		2
TOTALS	45	4

The mean total dose given to the 49 patients who were eventually completely cured was 2.28 grammes, to the 45 previously untreated patients 2.19 grammes, and to the 4 resistant patients 3.23 grammes. The patient who relapsed received 2.55 grammes.

*The relative total dose* — As before the doses have been calculated according to the weight of the patient, the 'relative' dose in this series being the dose per 100 lbs body-weight. The patients can be grouped according to the total relative dose administered, as follows —

Group	Ordinary cases	Resistant cases
15 to 199 gramme	3	
20 to 249 grammes	7	
25 to 299 „	17	
30 to 349 „	8	
35 to 399 „	6	1
40 to 449 „	1	1
45 to 499 „	2	1
50 to 549 „	1	
55 to 599 „		1
TOTALS	45	4

bladder, which constantly occurs on administration of this drug Plate LXXIV (b) Fifty milligrammes of cardiazol injected intravenously into a dog under experimental conditions always produce a well marked contraction of the bladder No such contraction, however, occurs on section of the dorsal cord This action is therefore a central effect

*Uterus*—Cardiazol causes a well marked increase in the tone of the intact uterus of both cats and dogs Plate LXXIV (c) Sometimes a preliminary depression has been noticed The uterine contractions were recorded by a modification of Barbour's method as described by Chopra and David (1927) Destruction of the medulla or section of the dorsal cord abolishes the stimulant effect It is also noticed that on the surviving uterus of a kitten, the drug has no stimulant effect but a relaxation is usually obtained Plate LXXV (b) The stimulant action of cardiazol on the uterus is also probably central in origin

### CONCLUSIONS

1 All animals do not react alike to cardiazol While fairly consistent results are obtained with cats, the effect on dogs is not so constant

2 The most prominent action of cardiazol is on the central nervous system It stimulates the central nervous system in general, and causes convulsions by acting on the medulla and spinal cord

3 It has been noticed that doses necessary to produce stimulation of the heart and respiration have also a tendency to cause muscular twitchings and convulsions

4 Cardiazol greatly stimulates respiration, not only the rate but also the amplitude being increased This effect is more or less constant

5 The stimulation of the heart obtained with cardiazol is transient, and the doses necessary to produce this effect seem to be too large compared with the weight of the animals used, being almost the same as the advertised therapeutic doses for human beings

6 No permanent damage to the heart is observed even after massive doses

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The drug is comparatively innocuous as in a series of unselected 70 patients only 4 died, in Dr Foster's series, here reported, there were 4 deaths amongst 89 patients. The death-rate in the two series is, thus, just over 5 per cent.

In this series of 70 patients 64 were discharged as cured, of these 49 are known to have remained in good health for a period of 6 months or more, and one relapsed.

In the series of 45 previously untreated patients whose subsequent history is known the mean number of injections administered was 11.04, the mean of the total actual dose was 2.19 grammes, and of the total relative dose 3.00 grammes per 100 lbs body-weight of patient, amongst these 1, or 2.2 per cent, relapsed.

#### ACKNOWLEDGMENTS

My thanks are due to Dr Percy Foster for placing the whole of his very carefully kept case notes at my disposal, to Rai Dr U N Brahmachari Bahadur for a generous supply of urea-stibamine, and to the Union Drug Co, Calcutta, for a similar supply of their brand of this preparation.

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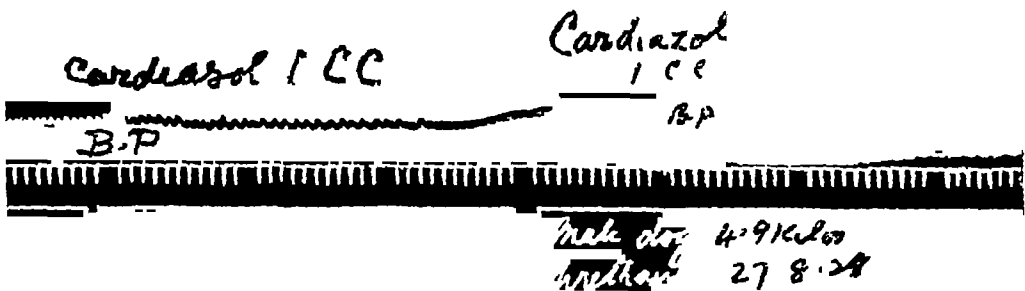
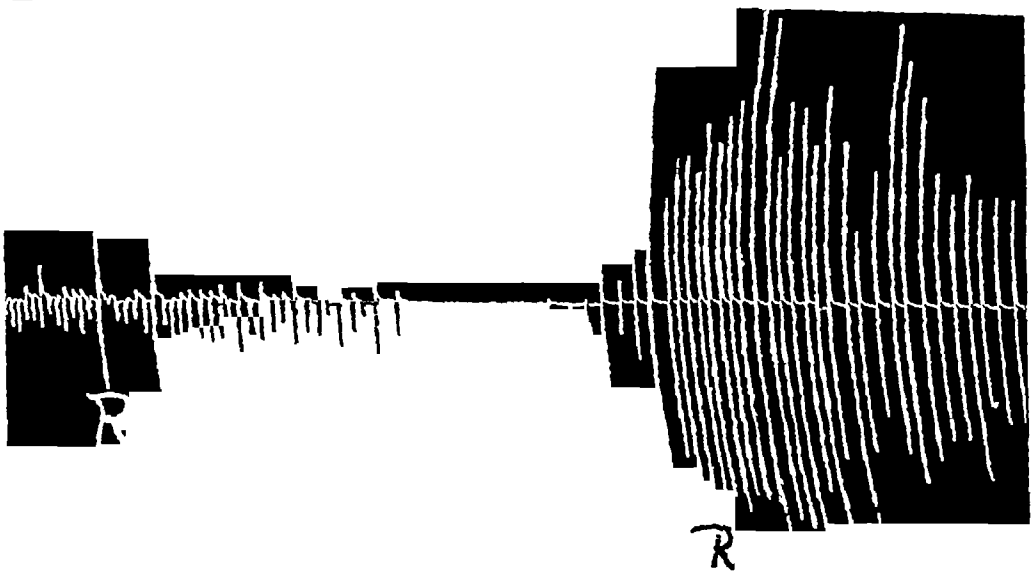
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#### APPENDIX

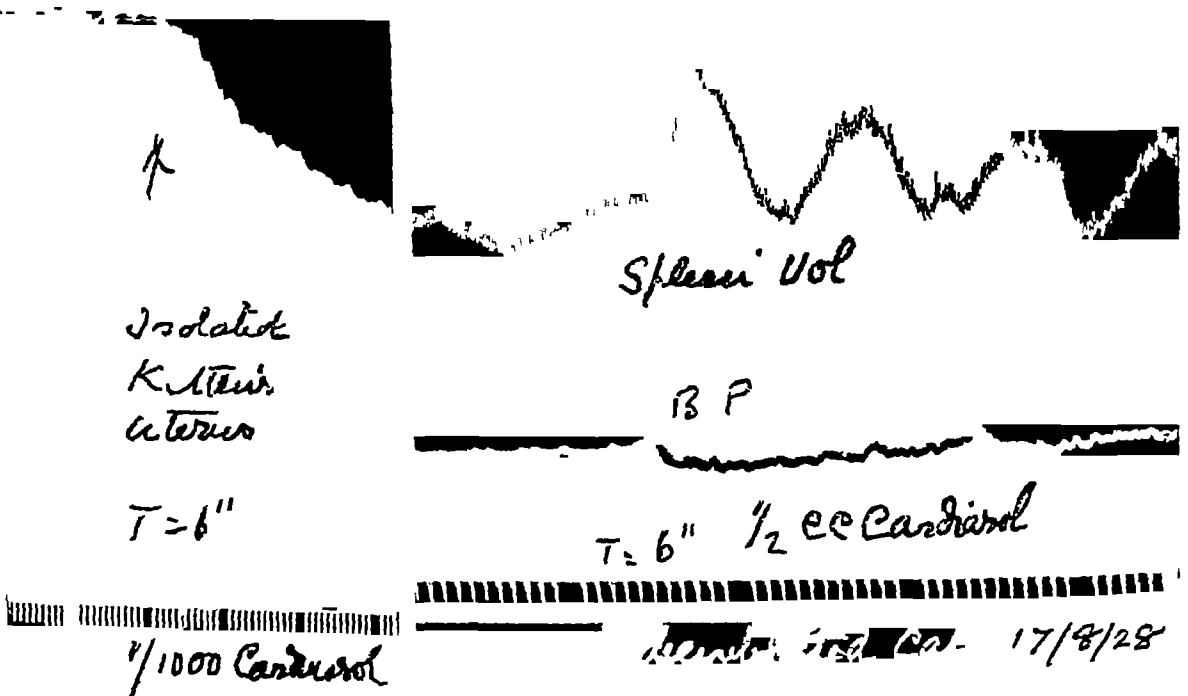
##### DR FOSTER'S RESULTS

Cured	81	Death rate	4.60 per cent
Relapsed	2		
Died during treatment	4	Relapse rate	2.41 "
No history	2		
Total cases treated	<u>89</u>		

Of the cured patients 8 had previously proved resistant to treatment by sodium antimony tartrate.



(a) Great depression of respiration of dog by morphine, stimulated by repeated injection of 100 mg cardiazol



(b) Isolated uterus of kitten—

(c) Decerebrated cat Blood pressure and spleen volume

# THE PENTAVALENT COMPOUNDS OF ANTIMONY IN THE TREATMENT OF KALA-AZAR

## Part V.

### STIBAMINE GLUCOSIDE (NEOSTAM) AN ANALYSIS OF THE TREATMENT IN 57 CONSECUTIVE CASES \*

BY

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*Officiating Director, Kala-azar Commission*

[Received for publication, October 1, 1928]

*The preparation*—This is another preparation with para-amino-phenyl-stibinic acid as a basis, it is described as a nitrogen glucoside of sodium para-amino-phenyl stibinate

It was first prepared by Dr Henry of the Wellcome Chemical Research Laboratories, has now been placed on the market under the name Neostam. It was first used clinically by the writer (Napier, 1925), who treated a series of 10 cases of kala-azar with a sample supplied by Dr Henry at the suggestion of Dr C M Wenyon, the Director of the Wellcome Research Bureau. It has subsequently been used by other workers in the treatment of kala-azar and other tropical diseases (Greig and Kundu, 1925, Hodgson, 1925, Struthers, 1927). The antimony content is about 30 per cent. It is supplied in the form of a light brown powder in sealed (and apparently nitrogen-filled) ampoules, dissolved in sterile distilled water it makes a sterile solution, clear and of a light brown colour. In the first few cases a 4 per cent solution was used, subsequently a 5 per cent solution was used. The injections were always given intravenously.

*Relative toxicity*—The relative toxicity of the compound is particularly low. A very large series of toxicity experiments was carried out in London. The toxicity of different batches of the compound differed slightly, but in no instance did a dose of 10 grammes kill more than half the mice to which it was administered,

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\* An investigation carried out under the Endowment Fund, Calcutta School of Tropical Medicine and Hygiene



0.05 cc of formalin were mixed and the time of gelation was noted. The pH of the resulting mixture of serum and buffer is, however, different from the pH of the buffer. Accordingly we measured the pH of such mixtures using buffers of different pH values. From these we can get an idea of the change of pH in the buffer caused by the addition of an equal volume of serum. As the quantity of serum from one patient was not sufficiently large, we could not perform the experiments on gelation and pH with the same serum. We, therefore, decided to make observations on a number of kala-azar sera to see the variations of pH in this mixture. In all our gelation experiments, we had to content ourselves by giving the pH of the buffer used for mixing.

TABLE I

*pH of mixtures of kala-azar serum and buffers of different pH values*  
(By the hydroquinhydrone method)

0.5 cc serum was mixed with 0.5 cc buffer of pH	pH OF THE RESULTANT MIXTURE					Mean
	1	2	3	4	5	
6.0	6.52	6.53	6.48	6.56	6.58	6.53
6.4	6.72	6.74	6.75	6.70	6.74	6.73
6.8	6.92	6.93	6.95	6.93	6.96	6.94
7.2	7.27	7.30	7.25	7.27	7.25	7.27
7.6	7.42	7.45	7.46	7.49	7.44	7.45

The pH value of mixtures of serum of buffers of pH value greater than 7.6 cannot be determined by the above method, as the quinol is oxidized to quinone in these pH values.

The time which elapsed after addition of formalin to produce a gel which does not flow when the tube was inverted, was taken as a measure of the progress of gelation. In a previous paper (10), we referred to the effect of pH on gelation but no details were given.

Table II gives the results of our experiments on gelation time at different pH values. The reliability and accuracy of this method of measuring the progress of gelation has been tested by repeating the experiments on the same serum, thus in one set, 0.05 cc of formalin was added to a mixture of 0.5 cc of serum and 0.5 cc buffer of pH 7.2. The times of gelation in two experiments were 15 and 16 minutes respectively. In another set, 0.1 cc of formalin was added to 1 cc of serum, in two experiments, the times of gelation were 25 and 30 seconds respectively. In all these experiments, Merck's formaldehyde was used.

*Results of treatment*—The immediate results of treatment of the 57 patients was as follows —

Discharged as cured	53
Failed to respond to treatment	2
Died during the course of treatment	2
	—
	57
	—

*The failures*—One was a child aged 9 years who had previously been given a full course of Stibosan but had relapsed. She was given 25 injections, amounting to a total of 3 125 grammes actual dose and 8 8 grammes relative dose, without showing any improvement. She subsequently had two further courses of treatment without improving and eventually died. The other was an Anglo-Indian woman who had previously been given a full course of urea-stibamine without improving. She received 3 2 grammes of Neostam in 15 injections without showing any improvement.

*The deaths*—The patients were both very debilitated when they were admitted. The first remained in an asthenic condition but appeared to be showing slight improvement when 24 hours after the 8th injection he died suddenly. The other patient was given small doses, not more than 0 15 gramme, but she continued to progress downhill and died 48 hours after the 6th injection.

*The patients discharged as cured*—Of the patients discharged as cured we have been able to get into touch with 44, of these, 35 have remained in good health for a period of six months or more, and 9 have relapsed.

*The number of days under treatment*—The mean number of days under treatment of the 53 patients discharged as cured was 25 75, of the 48 previously untreated patients the mean number of days was 25 40, and of the 5 resistant ones 29 20. Of the 35 patients that were eventually cured the mean was 25 71 and of the 9 that relapsed 28 11 days.

*Cessation of fever*—Of the 53 patients discharged as cured 6 were febrile and 3 were afebrile throughout the course of treatment. Of the rest the mean of the number of injections prior to the fall of temperature was 4 74, in the resistant cases it was 5 80, and in the case in which no previous treatment had been given it was 4 61, but this group included the 3 afebrile and the 6 cases in which fever was continuous throughout the whole course of treatment. Of the patients who were eventually cured 6 were febrile and 2 afebrile throughout, in one instance the time of fall of temperature was not noted, and in the remaining 27 the mean of the number of injections prior to the fall of temperature was 4 19. Of the patients that eventually relapsed one had no fever throughout and the mean of the number of injections prior to the fall of temperature of the remainder was 6 25.

suggests that development of opacity and gel-formation may not go hand in hand. Experiments with Cases 2 and 3 were done before Case 1 and, therefore, we had not the occasion to study in detail the process of development of opacity. But though these two classes of sera differ in respect of the pH value of minimum time of gelation, they exhibit similar properties in that the reaction is retarded on either side of the pH giving minimum time of gelation. What is to be noticed more is the fact that the reaction with both these classes of sera is retarded more on the acid side than on the alkaline side. In fact in Case 1 the time of gelation seems to be constant when buffers of pH 8.0 and 8.5 are taken. The reason why these sera show their minimum time of gelation at the two pH values 6.94 or 7.27 (see the pH, Table I) is not quite clear, but the existence of a minimum time of gelation at a particular pH value for a serum shows that this pH value is the iso-electric point of the protein which is responsible for gel-formation. In a previous paper (*loc cit*), the authors observed that the iso-electric points of globulin and albumin in the serum were 5.5 and 4.0 respectively. The globulin whose iso-electric point is 5.5 cannot, therefore, be responsible for the formol-gel reaction in kala-azar. In the same paper we have shown that urea stibamine mainly precipitates euglobulin, and that this precipitation diminishes as the pH value is gradually increased above 5.5. On the other hand, formalin precipitates some protein more on the alkaline side. 0.05 c.c. of formalin, 0.05 c.c. of kala-azar serum and 1 c.c. buffer of different pH values were mixed. At the pH value where occurred the minimum time of gelation, we found also maximum of opalescence. These facts, therefore, suggest two possibilities.

Firstly, gelation and opacity are caused by two proteins, the iso-electric point of the 'opacity-protein' being 5.5 and that of the 'gel-protein' being about 7.0.

Secondly, formalin reacting with the protein whose iso-electric point is 5.5 converts it into a new protein-complex with an iso-electric point of near about the pH value 7.0.

It has been mentioned by different investigators that since there is an increase in the euglobulin content of the kala-azar serum, this is the protein with which formalin reacts. In support of their views they showed that if kala-azar serum is dialysed and the euglobulin is removed by precipitation, gel-formation ceases. But it is just possible that the protein whose iso-electric point is near about the point of neutrality has got similar properties as euglobulin in being precipitated on dialysis.

## II RELATION BETWEEN OPACITY AND GEL-FORMATION. EFFECT OF pH ON OPACITY IN THE GEL. NATURE OF PROTEINS RESPONSIBLE FOR GEL-FORMATION AND OPACITY

It has been observed in the course of our experiments that in many cases the development of opacity and the progress of gel-formation are sometimes simultaneous and the two processes cannot be distinguished. In some cases, however, the process of gel-formation precedes the development of opacity.

*The number of injections given*—The number of injections given in each case is shown below —

Number of injections	NUMBER OF PREVIOUSLY UNTREATED CASES		NUMBER OF RESISTANT CASES	
	Cured	Relapsed	Cured	Relapsed.
9	2	1		
10	19	3		
12	5	1	1	1
13	1		1	
14	1			1
15	5	1		1
16		1		
TOTALS	33	6	2	3

The mean of the whole series is 11.57 injections. The mean of the number of injections given to the 33 cured patients that previously had no treatment was 11.21 injections and to the 2 resistant ones 12.5. The mean of the number given to the 6 relapsing patients that had previously had no treatment was 12.17 and to the 3 resistant ones 13.67 injections.

*The actual total dose*—The patients can be divided into groups according to the actual total dose received, as follows —

Group	PREVIOUSLY UNTREATED PATIENTS		RESISTANT CASES	
	Cured	Relapsed	Cured	Relapsed
Less than 1 gramme	2			
1.0 to 1.49 "	3			.
1.5 to 1.99 "	4	1	2	.
2.0 to 2.49 grammes	10	3		1
2.5 to 2.99 "	13	2		2
3 grammes or over	1			.
TOTALS	33	6	2	3

to 10 minutes and 8 minutes respectively. The marked increase in the gelation time is, of course, due to a decrease in the concentration of the protein that brings about gelation. But the time of complete opacity, however, increases from 5 minutes to 7 minutes and 6 minutes respectively. In the case of pure serum, gelation preceded opacity, but by mere dilution either with conductivity water or physiological salt solution opacity preceded gelation. The reason for this reversing of the order of the processes of gel-formation and development of opacity by mere dilution of the serum should not only be sought for in the change in the proteins brought about by dilution, but also in the nature of the diluent. Because if we dilute the serum with an equal volume of a buffer of either pH 7.4 or pH 9.34, gelation again precedes opacity, and although the time of gelation has increased only by 2 minutes, the change in the time of complete opacity as we pass from pH 7.4 to 9.34 is more than 20 minutes. Moreover, by dilution of the serum with conductivity water the pH diminishes and, comparing our results when the buffers of pH values 7.4 and 9.34 were taken, we expect that the time of complete opacity should be lower in this case and we find that it is so, though the time is greater than that of pure serum. From this it is obvious that both change of pH and change of concentration of the particular protein in the serum affect the time of complete opacity.

It is possible, therefore, that similar to the minimum time of gelation, there exists a pH value at which the time of complete opacity is minimum. If both the processes, i.e., gel-formation and development of opacity, are caused by one and the same protein, then not only the pH at which there exists a minimum time of gelation ought to show minimum time of complete opacity, but also the variation of the times of complete opacity and gelation with pH should run parallel. If, however, the two processes are caused by two different proteins, then the time of complete opacity ought to be minimum at some other pH, and the variations of these two times with pH should not run parallel. In Case 1 in Table II we have complete opacity when a buffer of pH 6.0 is taken but no gel-formation, we thought that opacity and gel-formation might be caused by two different proteins. The manner in which the time of complete opacity will vary on dilution of the serum will then depend on whether opacity and gel-formation are caused by one and the same protein or two different proteins. In the former case at the pH of minimum time of gelation there should be minimum time of complete opacity. On either side then the pH will tend to make the time of complete opacity greater. Dilution of the serum as we have seen above will also tend to make the time of complete opacity longer. Whether opacity will precede gelation and whether the time of complete opacity will be greater or less than that of pure serum, will depend on the relative effects of pH and dilution. When the dilution is constant, the time of complete opacity should pass through a minimum at the same pH value as the minimum time of gelation. In the latter case, i.e., if the two processes are caused by two different proteins the variation will depend on the iso-electric points of the two proteins. For the sake of clearness let us suppose that the iso-electric point of one protein

*The cure rate*—As this series contains a comparatively large number of relapses, an opportunity is given for the calculation of the cure rate with various doses of the drug. For this purpose, as has been pointed out before, it is only fair to take into consideration the previously untreated patients, as resistant patients are selected patients. The 2 patients that proved entirely resistant to treatment had previously undergone treatment and had relapsed so that these need not be taken into consideration. We are thus left with 39 patients, 33 of whom were cured and 6 of whom relapsed. Looking at the table of actual doses it will be seen that the relapses occur amongst the higher dose groups and that the mean of the total dose of the patients that relapsed was actually higher than that of those that were cured. On the other hand it will be seen from the relative dose table that here the relapses were more frequent in the lower dose groups and that the mean relative dose that the relapsing patients received was only 2.42 grammes, as against 3.18 grammes per 100 lbs of body-weight which the cured patients received.

Of 5 patients receiving a relative dose between 1.5 and 1.99 gramme (mean 1.75), 2, or 40 per cent, relapsed.

Of 7 patients receiving a relative dose between 2.0 and 2.49 grammes (mean 2.25), 2, or 28 per cent, relapsed.

Of 11 patients receiving a relative total dose between 2.5 and 2.99 grammes (mean 2.74), 1, or 9 per cent, relapsed.

Of 16 patients receiving a relative total dose of over 3 grammes (mean 4.32), 1, or 6 per cent, relapsed.

*Complications and sequelæ*—In about half the cases vomiting occurred. In 23 cases it was noted as a prominent symptom and in a few instances the patient vomited after practically every injection.

In no case was jaundice reported as a sequel to the course of injections.

#### THE CRITERIA OF CURE

As in this series there are an unusual number of relapses, an opportunity is given for observing if there are any points about the clinical progress of the patients during treatment which suggest that a relapse is likely to occur.

*Cessation of fever*—In all the relapsing cases the fever fell to normal before the conclusion of the course of injections, the average number of injections prior to the fall of temperature to normal was 6.25. Amongst the patients that were discharged as cured, 6 had fever throughout the course of injections, but in the remainder the mean of the number of injections prior to the fall of temperature was 4.61. There is, therefore, little indication in the course of the temperature during treatment to indicate whether a patient is cured or not.

*Weight*—Seven of the nine relapsing patients gained in weight during treatment, the mean of the gain being 7.7 lbs. Of the rest 39 out of 43 gained in weight, the mean of the gain being 8.00 lbs. The mean of the change in weight of all the patients in each of these two groups was an increase of 5.21 and 7.20 lbs, respectively. As 7 of the relapsing patients gained in weight, some

## Type II case

0.05 c.c formalin when added to 1 c.c serum, produces a translucent jelly within 20 seconds and develops complete opacity within 1 minute 5 seconds

*Gelation preceded opacity*

In the following experiments, 0.5 c.c serum, 0.5 c.c of buffer of a definite pH value, and 0.05 c.c of formalin were mixed and the time of gelation noted —

pH of the buffer	Time of gelation	Time of complete opacity	REMARKS
5.5	45 mins	16 mins	Granular structure opacity precedes gelation
6.2	11 "	5½ "	Do
6.6	5½ "	3½ "	Opacity precedes gelation
6.8	4½ "	3 "	Do
7.2	5½ "	5½ "	Gelation and opacity simultaneous
7.4	6 "	7 "	Gelation precedes opacity
9.34	8 "	32 "	Do

*N.B.*—Similar results were obtained with five other sera of each type whose times of gelation and complete opacity were about the same as those of the sera given above

An analysis of the results contained in Table IV shows —

Firstly, that the pH at which there exists minimum time of gelation is the same as the pH at which the minimum time of complete opacity occurs

Secondly, that development of opacity and progress of gelation pass through a maximum near the point of neutrality and are retarded by increase of either hydroxyl or hydrogen-ion concentrations

Thirdly, that development of opacity is retarded more on the alkaline than on the acid side, whereas the progress of gel-formation is set back more on the acid than on the alkaline side

No attention has been paid by the previous investigators to these facts

The fact that the minimum time of gel-formation and the minimum time of complete opacity occurs at the same pH value in one and the same serum points to the conclusion that both these processes are caused by one and the same protein, but the absence of the parallelism between the rate of variations of the times points to the other alternative of two different proteins. The apparent anomaly, i.e., the reversing of the order of the two processes, arises from the fact that the rate of decrease of opacity is much greater than the rate of decrease of gelation on the alkaline side, whereas the rate of decrease of gelation is much more pronounced on the acid side than the rate of decrease of opacity. This is clearly shown in the graph in the following figure. The times are plotted against the pH value of the buffer solutions. It will be noticed from a

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nearer the iso-electric point of euglobulin, its solubility is less and precipitation is more favoured, with the consequent quick development of opacity. So, on the acid side, one factor favours the development of opacity, whereas the other one retards it, and as a result the development of opacity is not so much retarded as gel-formation. On the alkaline side, precipitation of euglobulin is less favoured because the pH values are far away from the iso-electric point of euglobulin and, therefore, development of opacity is much more retarded than gel-formation. Hence, it seems more probable that these two processes are caused by two different proteins—one a 'gel protein' and the other the 'opacity protein'.

### III EFFECT OF DILUTION WITH DIFFERENT DILUENTS

From experiments on the effect of dilution on gel-formation and opacity (*vide* Table III) it is clear that when the concentration of the protein is only halved, the time of gel-formation increases enormously but not so the time of opacity. Also we have shown (Table IV) that the rate of decrease of opacity is much less than the rate of decrease of gel-formation on the acid side. The diluent that will change the pH of the serum towards the acid side will, therefore, retard gel-formation more than the development of opacity and, therefore, opacity may precede gelation. We know from our previous experiments (*loc cit*) that the pH of kala-azar serum decreases when diluted with conductivity water, therefore, when the serum is diluted with an equal volume of conductivity water we may expect that opacity might precede gel-formation. Data in Table III corroborates our views, i.e., opacity precedes gel-formation, when the serum is diluted with equal volume of conductivity water. Table V gives the results of some of our experiments on gel-formation only when the serum is diluted with an equal volume of physiological saline solution.

TABLE V

0.5 cc of formalin was taken

Time of gelation

1 cc serum	0.5 cc and 0.5 cc normal saline
Case 1—30 seconds	8 minutes
Case 2—20 "	4 "
Case 3—16 minutes	No gel formation within 2 hours but when kept over night in the cool room there was a gel with opacity

The time of gelation in Cases 1 and 2 were 12 to 16 times longer than that of pure serum. We, therefore, expect jellyfication to take place within 3 to 4 hours in Case 3. The diluted serum in this case actually set when kept overnight. We have done many experiments on this point and they all corroborate these conclusions. Gel-formation is always retarded on dilution of the serum with an equal volume of physiological saline solution (0.85 NaCl). When the time of gelation of the original serum is under 1 minute, we will have always

workers obtained definite stimulation of the isolated frog's heart with a dilution of 1 in 5,000 and that of the rat with a 1 in 10,000 dilution. They also found that cardiazol stimulated the respiratory centre after morphine depression. Blume (1926) concluded that cardiazol increases the reflex irritability of the cord, the receptor portion of the arc being affected. Schoen (1926) opines that the drug is a stimulant of the spinal cord. Stross (1926) says that cardiazol does not increase the work of the normal heart but has a stimulating influence on a heart mechanically weakened. Strube (1927) also found that cardiazol might stimulate a depressed heart. Kessler (1927) read a paper on cardiazol at the 7th Congress of the Far Eastern Association of Tropical Medicine held in Calcutta in December of that year. He said that it was a stimulant of the cardiac muscle and of the respiration and that it was of great use clinically. These observations seem to have been confirmed by a number of other continental workers who have tried the drug clinically. Camp (1928) comes to the conclusion that cardiazol has no apparent effect on the normal heart, that it effects convulsions by acting on the medulla, that its action on the respiratory centre is not uniformly excellent, that the dilatation of the capillaries in the abdominal organs speaks against its use in collapse and shock and that the substance is not free from untoward by-effects although these may be transient in character. Barker and Levine (1928) found in a series of experiments that cardiazol did not have any beneficial effect on the cardio-respiratory mechanism. This was true in the normal animal and in states of depression, produced by quinidine, hæmorrhage and acid intoxication.

In view of the conflicting nature of the literature here presented and because of the fact that this drug is widely advertised in all the leading medical journals in India, a study of its pharmacological and therapeutic actions was deemed advisable. We have to thank, at the outset, the manufacturers and their local agents, Messrs Martin and Harris of Calcutta for having supplied us with sufficient quantities of the drug to carry out our experiments.

#### PHARMACOLOGICAL ACTION

*External*—Cardiazol solutions have no irritating effect on the skin. Even high concentrations like 1 in 10 have no antiseptic effect on bacteria.

*Central Nervous System*—Experiments were done on frogs, cats and dogs to elucidate the action of cardiazol on the nervous system. Twenty milligrammes of the drug injected into the ventral lymph sac of a frog cause the following symptoms. The respiration is stimulated and becomes hurried within about a minute. When disturbed, the animal does not hop about as usual but just crawls along. This 'latent' period is followed in about another 5 minutes by violent convulsions resembling those produced by picrotoxin. The convulsions persist after the cerebral hemispheres are removed, but disappear on destruction of the medulla. The site of action seems therefore to be in the medulla. Convulsions are also produced in cats and dogs. In the cat, the convulsions are preceded by a period of restlessness, during which respiration is hurried and peculiar jerky movements of the head, as if the animal is trying to snap at imaginary objects, are noticed. The

the time of gelation and, therefore, either opacity precedes gelation or both the processes are simultaneous. As the concentration of the protein that is responsible for opacity in the serum decreases, gelation tends to precede opacity and the difference in the two times will depend on the relative concentrations of the proteins in the serum and also on their buffer action.

*Why is the aldehyde test negative in early cases of kala-azar?*

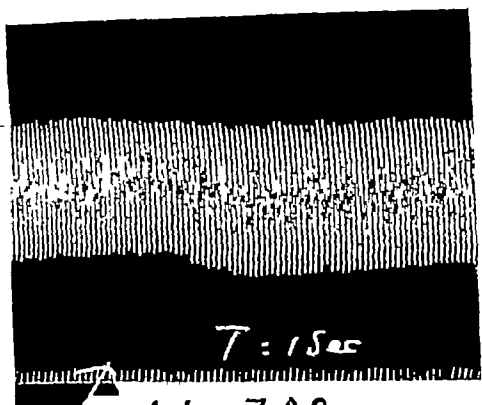
*Why in malaria, tubercle, and some other pathological sera is there gelation but not complete opacity?*

When the concentration of the protein that forms a gel with formalin is not high as in some early cases of kala-azar, advanced malaria and tuberculosis, etc., the times of gelation and complete opacity are much longer as compared to advanced kala-azar. The buffering action of the sera in the former groups of cases is comparatively higher than the latter, the time of complete opacity, therefore, will be still longer in the former group. That is the reason why the aldehyde test fails in early cases of kala-azar. There is also a possibility that very advanced cases of malaria, tuberculosis, etc., might give a positive aldehyde test when the concentration of the particular protein is sufficiently high. Brahmachari(11) has shown that there are definite non-kala-azar cases which give a positive aldehyde test and we have ourselves observed this in some cases of advanced leprosy. A difference in the relative concentrations of these two proteins of course explains these phenomena, but what we wish to emphasize is the fact that buffer action also plays a part in these processes.

We have referred to the early and advanced stages of kala-azar. When the duration of the disease is only 2 or 3 months, the aldehyde test is usually negative because complete opacity does not develop within the time limit set by the author of the test. From this it might appear that the time of gel-formation and opacity will depend on the duration of the disease. But taking a few cases of over 6 months duration, we have found that there is no relation between the time of the gel-formation and the duration of the disease. This will be seen from a perusal of Table VI.

TABLE VI

	Duration of disease	Time of gelation	Time of complete opacity
Case 1	9 months	50 secs	50 secs
Case 2	12 "	18 mins	
Case 3	6 "	20 secs	1 min 5 secs
Case 4	6 "	1 min	1 " 10 "
Case 5	18 "	2½ mins	8 mins
Case 6	6 "	3 "	11 "

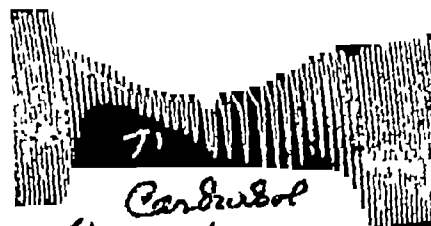


1 in 300  
Cardiazol  
Frog's Heart isolated  
3/17/29



1/50 Cardiazol  
Frog's Heart isolated

Isolated Frog's Heart  
4/17/29

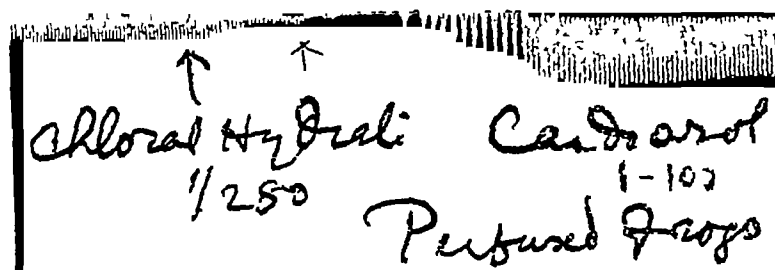


Cardiazol  
1/100  
T = 5 sec

(a) Isolated frog's heart 1 in 300 cardiazol

(b) Isolated frog's heart 1 in 50 cardiazol

(c) Tartar emetic 1 in 10 000 at arrow-mark Revival with cardiaz



Chloral Hydrate 1/250  
Cardiazol 1-100  
Perfused Frogs

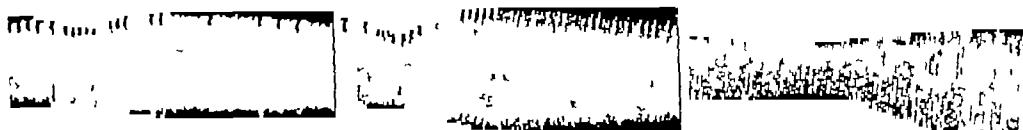


depressed by  
1/10000 Emetine  
1/1000 CARDIASOL  
T = 15 sec

(d) Perfused frog's heart Depressed by chloral hydrate 1 in 250 Revived by cardiazol 1 in 100

(e) Isolated frog's heart Depressed by emetine 1 in 10 000 cardiazol 1 in 1 000 at arrow

Perfusion of Isolated Kitten's heart



10 mg Cardiazol  
T = 6"  
Isolated Kitten's Heart

1 mg Cardiazol  
T = 6"  
Cardiazol 1/2 cc

(f) Cardiazol 1 in 1 000

(g) Cardiazol 1 in 500

(h) Cardiazol 1 in 200

(i) Cardiazol 1

(4) By simply diluting the serum with an equal volume of different diluents, we can reverse the order of the processes of gel-formation and development of opacity with formalin, i.e., in diluted serum, sometimes gelation precedes opacity and sometimes opacity precedes gelation

(5) These facts suggest either that gel-formation and opacity are caused by two different proteins or formalin reacting with euglobulin produces a marked change in its nature

(6) An attempt has been made to explain the aldehyde test for kala-azar on the basis of the above-mentioned facts

We have much pleasure in acknowledging the help and advice we have received from Lieut-Col H W Acton, I.M.S., and also from Prof J N Mukherji, D.Sc., of the University College of Science in the course of this research

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c

Plate LXXIII (a) There is no change in the rate of the heart The stimulation, however, is not persistent but disappears in a couple of minutes The effect is as noticeable in a decerebrated animal Plate LXXIII (b), and persists even after paralysis of the cardiac sympathetic nerve endings by ergotoxin Plate LXXIII (c) The stimulation is not due to the depression of the vagal nerve endings as it is still seen after atropine injections The action of cardiazol thus seems to be purely on the musculature of the heart No toxic effects are noticed on this organ even after repeated intravenous injections of 100 mg

*Blood-pressure*—The effect on the blood-pressure is not constant Fifty milligrammes given intravenously usually cause a slight fall of blood-pressure which is quite transient Sometimes there is no fall but a slight rise Doses of 100 mg invariably produce a fall of blood-pressure, which, however, is not very marked The variation in the blood-pressure seen in Plate LXXIV (a), is due to the onset of convulsions

Oncometric tracings of the spleen show a well marked increase in the volume as well as in the rhythmic movements of this organ Plate LXXV (c) The kidneys also show a rise of volume Plate LXXIV (a) Limb plethysmographic records did not show constant results, but there was a slight but gradual increase in volume in one case Plate LXXIV (a) The intestinal volume also increases, indicating dilatation of the splanchnic blood vessels The fall of blood-pressure is, therefore, due to this vaso-dilatation Perfusion of the blood vessels of the pithed frog with dilutions of cardiazol does not cause any appreciable change in their calibre Probably the vaso-dilatation is of central origin

*Respiration*—It has been mentioned that injection of cardiazol into the lymph sac of the frog causes the breathing to be hurried The same result has been noticed in animals like cats and dogs Intravenous injections of 50 mg of cardiazol to these animals anaesthetized with urethane invariably produce a very marked stimulation of respiration, both the rate and the amplitude being increased Plate LXXIV (b and c) This stimulant effect is also seen after depression of respiration by morphia Plate LXXV (a) shows a well marked stimulation of respiration after it was almost stopped by the toxic action of morphia on the respiratory centre Cardiazol therefore appears to have a direct stimulant action on the respiratory centre Camp (1928) found that 20 mg of cardiazol injected into the fourth ventricle after respiration had been stopped, caused a marked stimulation of the respiratory movements

*Intestines*—No change in the movements of the gut is noticed, as recorded by the balloon method

*Gemto-urinary system*—The kidney volume has already been shown to be increased

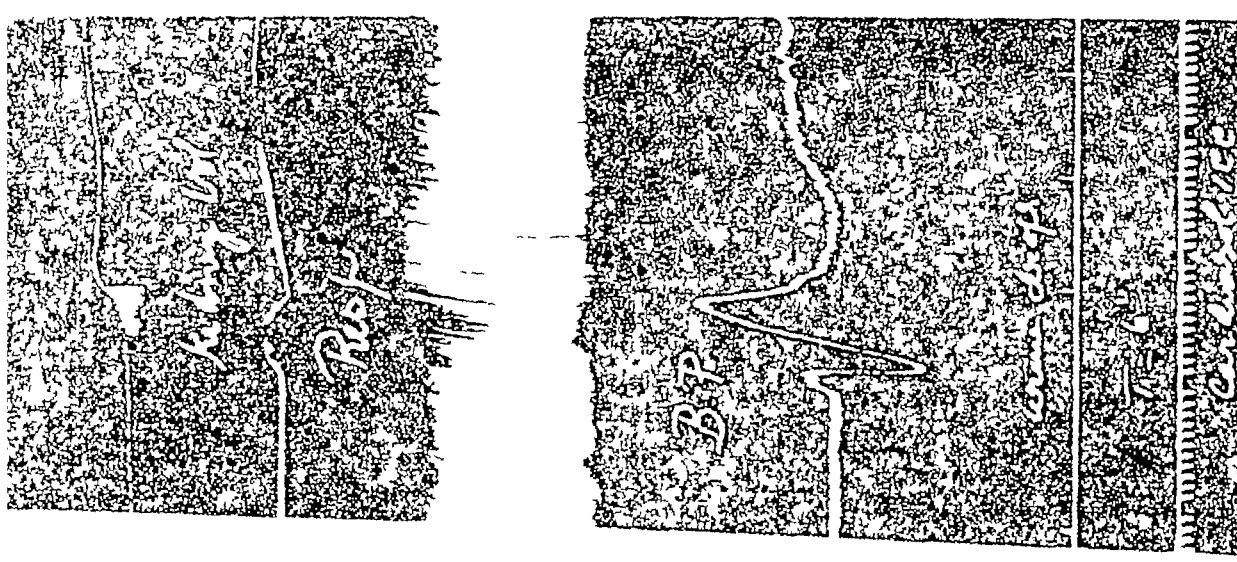
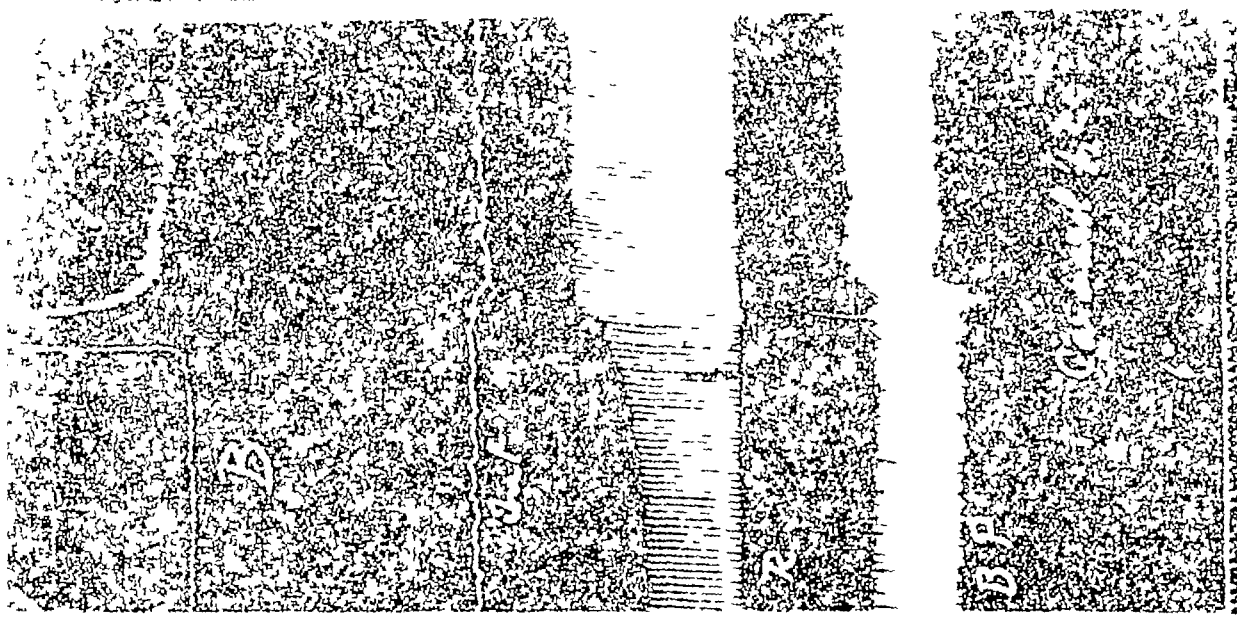
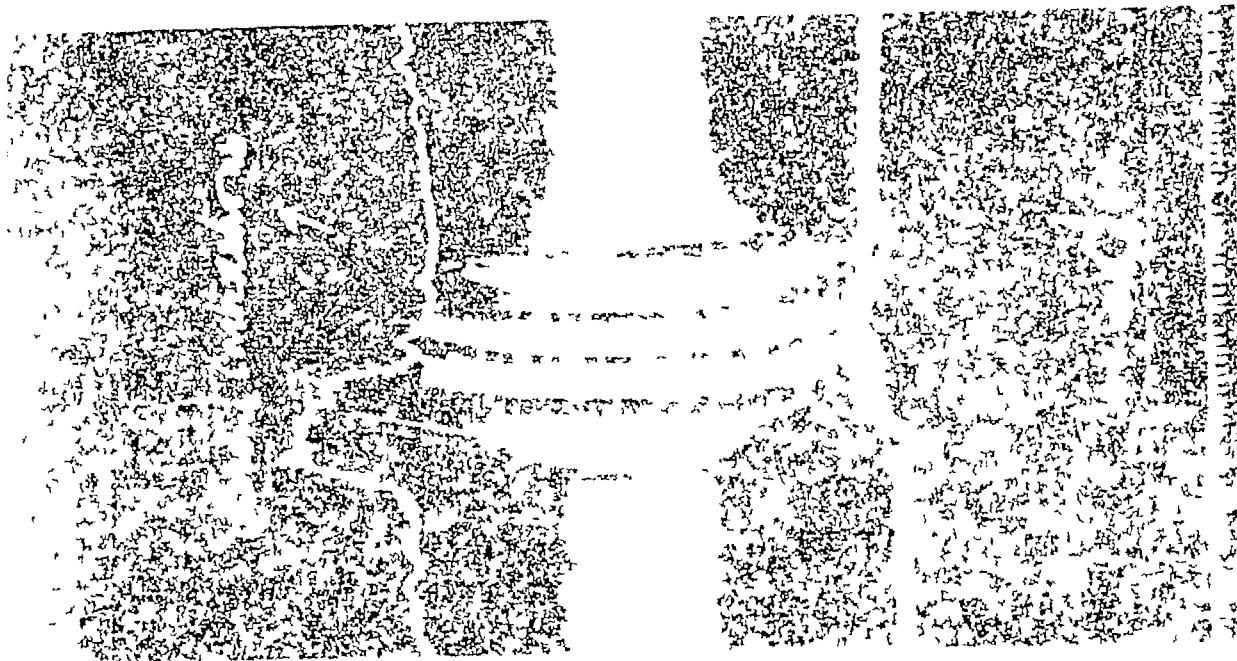
No definite information could be obtained about the influence of cardiazol on the urinary secretion In one case Plate LXXIV (a), it produced a slight increase in the number of drops of urine as recorded by the drop counter But as in this case the cannula was placed in the bladder and not in the ureter, the apparent increase in secretion might have been due to the contraction of the

viscosity of various proteins, because for the same amount of protein, different viscosimetric values have been obtained. The explanation for this observation has been furnished by the experiments of Heyder and Rohrer (1915) who showed that the proteins of the serum consist of two substances of different viscosity, albumin and globulin, the former being less viscous than the latter. It may, therefore, be expected that dilute solutions of the same total protein content will have lower or greater viscosity as they are richer in albumin or in globulin respectively. Heyder and Rohrer (*loc cit*) claim to have established a definite relation among the three factors, viscosity, protein percentage and albumin-globulin mixture. If their contentions be true, then we may expect to have a qualitative idea of the relative proportions of albumin and globulin in different pathological sera from values of viscosity, at the same time these determinations may furnish some definite values of viscosity for different types of pathological sera, as has been claimed by Bircher and his co-workers to be the case in syphilis, cancer and tuberculosis, in secondary anæmia and in peritoneal infections by Ochlecker (1910), Simon (1911) and Sussenguth (1912) and in pneumonia by Ch. Achard, Touraine and St Girons (1912).

### *Experimental*

The applicability of ordinary methods of determining viscosity in true solutions to that of colloids, as is well known, is not free from objection. The difficulty consists in the theoretical interpretation of the 'effective' resistance to the flow. In our experiments we compared the viscous resistance, as measured by the ordinary flow methods, of normal and pathological human sera with a view to compare the rates of flow under similar conditions. The same viscosimeter was used in all the measurements.

If the times of flow for the same volume of two different liquids are determined, the relative viscosity of the liquids is given by the expression  $\frac{n_1}{n_2} = \frac{t_1 d_1}{t_2 d_2}$  where  $n_1$ ,  $t_1$  and  $d_1$  are the viscosity, time of flow and the density of one liquid and  $n_2$ ,  $t_2$  and  $d_2$  are the corresponding quantities for the second liquid. If the absolute viscosity of one liquid (e.g., water) is known, the absolute viscosity of the other liquid can be calculated. For comparative purposes, the relative viscosity was worked out by taking that of the water as unity. A micro-viscosimeter of the Ostwald type was used and with it the time of flow of one c.c. of serum under observation was measured and the rate compared with that of water. All measurements were taken at the room temperature which varied from 25°C to 28°C. Densities were simultaneously determined. The temperature co-efficient of viscosity  $\frac{dn}{dT}$  is, however, not altogether negligible. We have expressed all our results at 25°C, reducing the values found at other temperatures to those of 25°C. The co-efficient has been found to be approximately 1.5 per cent within the above range of temperatures. Bircher and Macfarland (*loc cit*) have similarly expressed their results at 20°C. Before each set of





## DISCUSSION

A study of the results given in the above table will show that the relative viscosity of pathological sera we have studied is generally greater than that of the normal sera. The highest value has been obtained with sera from the blood of kala-azar patients. In the case of leprosy there is a wide variation in the viscosity. Though in some cases these values are decidedly higher than that obtained with serum from the blood of healthy persons, in the majority of cases studied the variations were about the same as in healthy individuals. It is also to be noted that in some cases of leprosy there is a slight decrease in relative viscosity from the normal value. It is well known that about 60 per cent of lepers are syphilitic, so that it is not surprising to find a relative increase in viscosity of serum from the blood of some of these patients, but the slight decrease observed in a few cases is worthy of notice. The viscous resistance of such systems is not independent of the size and shape of the dispersed substance and therefore the viscosity will depend on the amount of water associated with one molecule of protein\*. The hydration factor or the relative number of molecules of water

\* To understand the true significance of these variations in relative viscosity, we must turn our attention to the theoretical formulæ deduced in a general way for simple systems of dispersoids by A. Einstein (1906), M. V. Smoluchowski (1916), W. R. Hess (1920) and in a restricted form by Hatschek (1911). Einstein deduces from fundamental equations of hydrodynamics the relation

$$\eta' = \eta (1 + 2.5f)$$

in which  $\eta' =$  viscosity of suspension,  $\eta =$  viscosity of dispersion medium, and  $f$  is the ratio aggregate volume of solid particles/volume of suspension. The viscosity of a suspension accordingly increases in a linear ratio with the percentage of suspended solid and is independent of the degree of dispersity, since the diameter of the particles does not appear in the equation. That a linear formulæ cannot be considered as anything more than a first approximation, has been proved by the experimental researches of Hatschek and theoretical considerations of Smoluchowski. The formulæ which fits in with the results on viscosity of a suspension of red blood cells is that of Hatschek (*loc cit*). The viscosity  $\eta'$  of the emulsion is given by the formulæ

$$\eta' = \frac{\eta}{1 - \sqrt{f}}$$

where  $\eta$  is the viscosity of the continuous phase and  $f$  the ratio volume of disperse phase/total volume. If  $\eta'$  is known, the value of  $f$ , the phase volume ratio is

$$f = \left( \frac{\eta' - \eta}{\eta} \right)^2,$$

and this volume ratio can then be compared with the known concentration by weight. From these considerations we can calculate the number of water molecules associated with one molecule of protein from the results on euglobulin and pseudoglobulin obtained by H. Chick (1914). The figures are abnormally high, compared to corresponding ratios in true solution, i.e., of cane-sugar. S. Arrhenius (1916) applies to the viscosity for the proteins quoted above his empirical formulæ

$$\log \eta = \theta c$$

where  $c$  is the molecular concentration. When the formulæ is applied to viscosities of protein solutions, it fits in with remarkable accuracy. The hydration factor, calculated with the help of this formulæ, has been given above.

# ON THE CAUSATION OF FORMOL-GEL REACTION IN KALA-AZAR

## Part I.

BY

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## INTRODUCTION

NAPIER (1921) showed that when one drop of formalin is added to 1 c c of serum from the blood of a kala-azar case, a white opaque jelly is usually obtained(1, 2, 3, 4) This gel-formation has been ascribed by many authors to the increased euglobulin content of the serum from the blood of a kala-azar patient(5, 6, 7) Lloyd and Paul (1928), however, partially accept this view and they suggest that gel-formation is associated with a special kind of euglobulin present in the serum of kala-azar cases, as also with the albumin and pseudoglobulin fraction(8) None of these authors appear to have studied in detail the conditions on which this reaction depends The present investigation was undertaken with the idea of studying the physico-chemical conditions on which this reaction depends in order to throw some light on the nature of the reaction, as well as on the nature of the substances taking part in it

## I EFFECT OF PH ON GEL-FORMATION ARE GEL-FORMATION AND OPACITY CAUSED BY TWO DIFFERENT PROTEINS?

It was pointed out in a previous paper(9) that the pH of the medium affects the process of gel-formation It was, therefore, thought desirable to study the process of gel-formation in serum from kala-azar cases by varying the pH of the medium 0.5 c c of serum and 0.5 c c of buffer of a definite pH value and

Finally, we would like to point out that there appears to exist a relation between the physico-chemical properties of blood serum—viscosity, surface tension and buffer action. A serum which shows higher viscosity, exhibits diminished buffer action and lower surface tension, as would appear from the following rather small number of observations. Each reading given in the following table is a mean of five observations —

TABLE

Disease	Relative viscosity	Surface tension in dynes per cm	pH of 0.4 cc of serum and 0.6 cc. of 0.1 N HCl
Kala-azar	2.28	55.0	6.70
Syphilis ..	1.75	58.8	6.75
Leprosy	1.56	59.1	6.88

If these observations are corroborated by further experiments, it may lead us to a greater insight into the reactions of the sera against diseases.

## SUMMARY AND CONCLUSIONS

(1) Relative viscosities of normal and some of the pathological sera have been measured. The highest values have been observed in kala-azar sera. Syphilitic sera also show an increase in this value. Serum from the blood of lepers shows divergent values.

(2) The increase in viscosity has been attributed to a relative increase in the globulin content of serum, the cause of exceptionally high values has been attributed to a relative increase of euglobulin.

(3) Advanced kala-azar and tubercular patients show such abnormally high values, it is suggested that in both these types of sera there is more euglobulin.

(4) The buffer action of a serum that shows higher viscosity and lower surface tension is diminished, the buffer action of serum from the blood of leprosy cases (not contaminated with any other disease) is higher than those of syphilitic and kala-azar sera.

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TABLE II  
Formalin added, 0.05 c.c  
Phosphate buffers used    Temperature 30°C to 31°C

pH of the buffer	Approximate pH of the resulting mixture	Case 1	TIME OF GELATION		Case 4
			Case 2	Case 3	
6.0	6.53	No gelation within 50 mins		Not within 2 hours	
6.4	6.73			27 mins	2 mins 10 secs
6.5		18 mins			
6.6			More than 30 mins	20 mins	1 min 15 secs
6.8	6.94	11 mins	22 mins	10 "	1 " 20 "
7.0			24 "	7 "	1 " 10 "
7.2	7.27	13 mins	More than 30 mins	5½ "	1 " 2 "
7.4	.	14 "			1 " 5 "
7.6	7.45	18 "		9 mins	1 " 8 "
8.0		20 "			
8.5		20 "			.

From the results given in Tables I and II, it is clear that the hydrogen-ion concentration of the medium has a marked effect on the gelation time in kala-azar serum. Near about the point of neutral reaction of the solution the time of gelation is minimum, but increase of both hydroxyl and hydrogen-ion concentrations retards this reaction.

It is to be noticed that in two instances the minimum time of gelation occurred when the buffer of pH value 6.8 is taken and, in the other two, this minimum shifts to a buffer of pH value 7.2. At first we suspected that this might be due to our error of observation. But repeating these experiments on several samples of sera, we found that in some cases the minimum time of gelation is, undoubtedly, situated at the pH of the mixture of the serum and buffer of pH 6.8, while in others it is at the resulting pH of the serum, when a buffer of pH 7.2 is added. Hence, we thought it desirable to make more detailed observations on opacity and gel-formation. In Case 1, the mixture of buffer and serum was perfectly opaque by the time it set. In the case of the buffer of pH value 6.0 when formalin was added, there was absolutely no opalescence at first, but within 50 minutes the serum was perfectly opaque without any gel-formation. This, of course, shows that opacity might develop on addition of formalin without gelation, and it also

# THE ADHESION PHENOMENON IN FILARIASIS A PRELIMINARY NOTE

BY

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THE adhesion phenomenon, otherwise known as the Rickenberg reaction, has been chiefly studied in trypanosomiasis, and in some spirochætal and leptospiral infections. The phenomenon consists essentially in the adhesion either of leucocytes or blood platelets or even of dead bacteria to the parasite in the presence of its homologous immune serum.

During an investigation into the biological differences between the microfilariæ of man and animals, we came across a similar phenomenon with *Microfilaria bancrofti*. The action of sera on microfilariæ from cases of elephantiasis was being investigated when it was found that in such mixtures the leucocytes tended to adhere to the microfilariæ along their entire surface, while in mixtures with normal sera no such adhesion was noted. Many sera have been tested since and the results generally confirmed.

## TECHNIQUE

For the microfilarial suspension 8 c.c. of blood are obtained at night from a vein of a person harbouring the microfilaria and mixed with 2 c.c. of 2 per cent citrated saline solution to prevent clotting. The citrated blood also serves as a leucocyte suspension. It is not necessary to separate the microfilariæ and the leucocytes from the red cells. Equal volumes of each serum to be tested and the microfilarial suspension are taken in a sterile capillary pipette and thoroughly mixed in small sterilized glass vials. These are plugged with cotton and incubated

Since perfect opacity in the gel is an essential factor in the test for kala-azar cases and since we know that sera from the blood of patients suffering from some other diseases form gels with formalin with more or less opacity, we studied the processes of gel-formation and development of opacity in detail in order to find out if any relation existed between them

We obtained very interesting results with a serum we collected from a kala-azar case. When 0.05 cc of formalin was added to 1 cc of serum, the serum set to a jelly within 30 seconds with a moderate amount of opacity, i.e., the jelly was translucent. Within 5 minutes the serum became perfectly opaque. We, therefore, used this serum for this particular investigation. The results of our experiments are recorded in Table III. The time of complete opacity has been taken to be that which elapsed after addition of formalin for the serum to form a gel which is opaque through both reflected and transmitted light.

TABLE III

0.05 cc of formalin was added

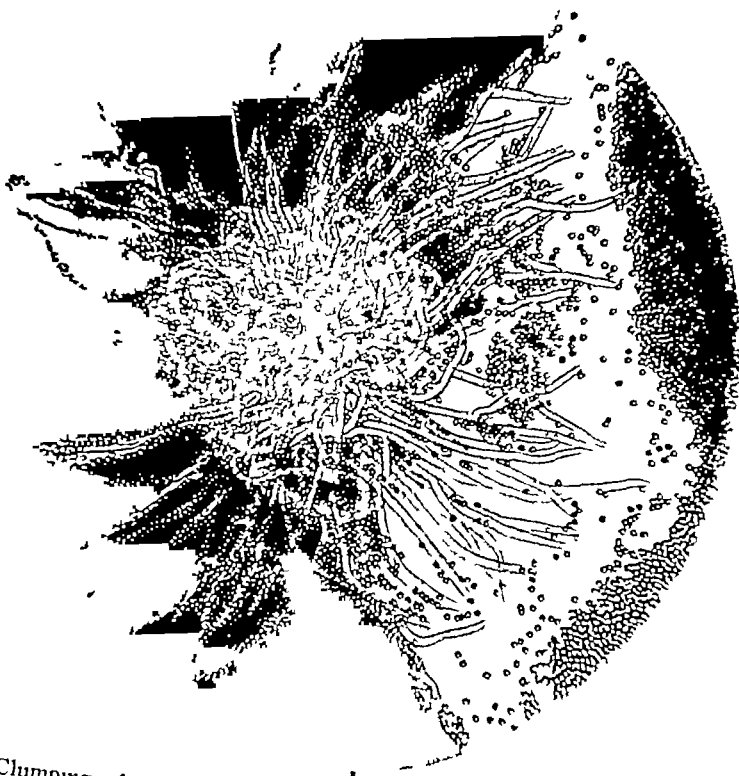
1 cc of serum set to a jelly within 30 seconds and was completely opaque within 5 minutes

No	Serum	Diluent	Time of gelation	Time of complete opacity	REMARKS
1	0.5 cc.	0.5 cc of conductivity water	10 mins	7 mins	Opacity precedes gelation
2	0.5 cc	0.5 cc physiological saline solution	8 "	6 "	Do
3	0.5 cc	0.5 cc. phosphate buffer of pH 7.4	6 "	9 "	Gelation precedes opacity
4	0.5 cc.	0.5 cc disodium hydrogen phosphate of pH 9.34	8 "	More than 30 mins	Do
5	0.5 cc.	0.5 cc formalin of pH 2.8	Not within 3 hours	Not within 3 hours	At the end of 3 hours the serum was clear through transmitted light, slightly opalescent, set to a translucent jelly when kept overnight

*NB*—The table given here contains results of only one particular serum, but similar results were obtained from five others when their times of gelation and complete opacity are about the same as that of the serum taken here.

It becomes apparent from the perusal of this table that on dilution of the serum, the time of gelation always becomes longer as well as the time of complete opacity. When the serum is diluted with an equal volume of conductivity water or physiological salt solution, the time of gelation is increased from 30 seconds

PLATE LXXVII



Clumping of microfilariae *in vitro* no adhesion seen

(responsible for gel-formation) is about 7.0 and that of the other (responsible for opacity) is 5.5. We see then that above pH 7.0 and below pH 5.5, variation of pH will tend to make both the times of opacity and gelation longer. But between the pH values 7.0 and 5.5 decrease in pH makes the time of complete opacity shorter but dilution makes it longer. Depending on the magnitudes of these two effects, the actual time will be determined. When the dilution is constant, decrease in pH between 7.0 and 5.5 will decrease the time of complete opacity. Whether the time of complete opacity should be greater or less than that of pure serum cannot be foretold without an accurate knowledge of the effects of pH and dilution. On the other hand within this range of pH values, the time of gelation will be greater for a decrease in pH. The effect of dilution is always to make it longer. Hence, the time of gelation will always increase within this range of pH values and there is, therefore, a possibility that opacity might precede gelation when the pH value is decreased. Since with Case 1 in Table II we have complete opacity when a buffer of the pH value 6.0 is taken but no gel-formation, we thought that opacity and gel-formation might be caused by two different proteins. But the results on dilution in Table III can be explained on the basis of either of these two assumptions. To decide which of these two assumptions is correct, we determined both the time of gelation and the time of complete opacity at different pH values. The results are given in Table IV. As the quantity of serum obtained was not sufficient to perform experiments at all ranges from 5.5 to 9.3, particular pH values suitable for the purpose were selected.

TABLE IV

0.05 c.c formalin when added to 1 c.c of serum produce a completely white jelly within 20 seconds. Times of complete opacity and gelation cannot be distinguished.

In the following experiments, 0.5 c.c of serum and 0.5 c.c of buffer were mixed and 0.05 c.c of formalin added —

pH of the buffer	Time of gelation	Time of complete opacity	REMARKS
6.4	2 mins 10 secs	55 secs	Opacity precedes gelation
6.6	1 min 45 "	45 "	Do
6.8	1 " 20 "	45 "	Do
7.0	1 " 10 "	45 "	Do
7.2	1 " 2 "	40 "	Do
7.4	1 " 5 "	50 "	Do
7.6	1 " 8 "	55 "	Do



D Sera from healthy persons

E Sera from convalescent cases of non-filarial fever, but showing a high leucocytosis

Groups D and E served as controls Only a few of these could be examined for microfilariae in blood They were all negative

The results obtained are summarized in the following table The details of cases in Group A are given in the appendix

Group	Number examined	Number showing positive reaction	Percentage positive
A	32	25	78
B	10	0	0
C	3	1	33
D	13	3	23
E	4	0	0

The results may be analysed as follows —

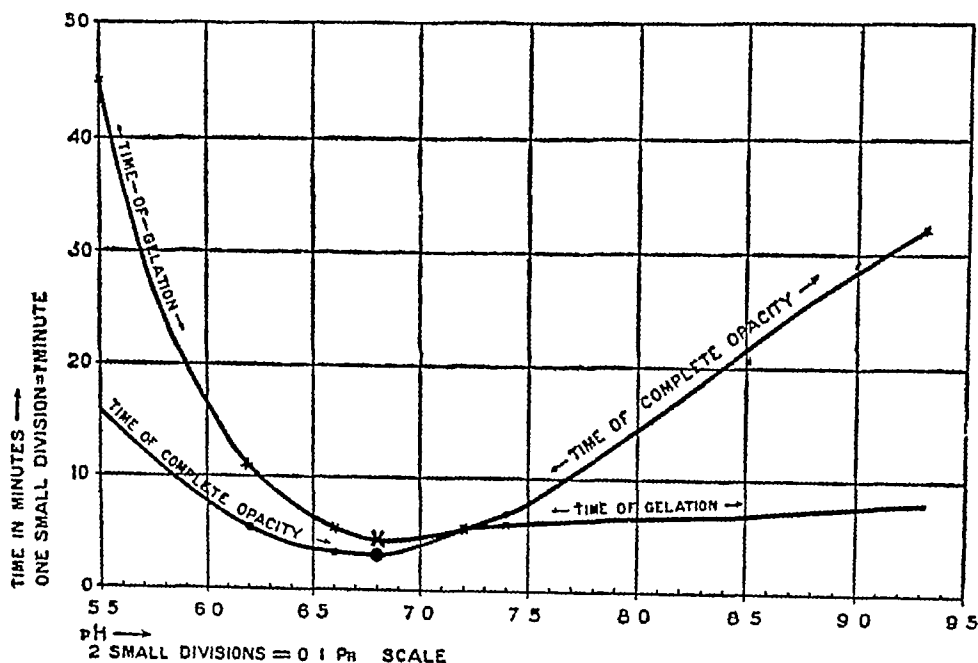
(1) The majority of sera from elephantiasis cases (78 per cent) gave a positive reaction Microfilariae were not seen in any of these bloods

(2) The sera from cases harbouring microfilariae, as well as those from apparently healthy persons, did not show the reaction with four exceptions, one in Group C and three in Group D Three of these had lived for a long time in highly filarial areas—Cochin and Saidapet One (Group C) had an attack of chyluria with fever 16 years ago, but has been free from any signs and symptoms of filariasis since then No microfilariae were found in the blood at any time The second had an attack of funiculitis two years previously The third had never shown any signs of filarial disease and the history of the fourth is not available

### DISCUSSION

It appears from the foregoing results that in the case of elephantiasis sera, where microfilariae are not found in the blood, some anti-body is present, which is absent in other sera This anti-body brings about the adhesion of leucocytes to the microfilariae and thus helps in their destruction The absence of microfilariae in elephantiasis cases can thus be explained If this reasoning is correct, one ought not to find this anti-body in sera in Group B, where microfilariae are found in blood The absence of this phenomenon in those sera supports this hypothesis The question whether this anti-body is due to the presence of the parent worm living or dead, or to the natural death of microfilariae which would then serve as antigen in its production is yet to be determined

comparison of Table I and the graph that the absence of the parallelism between the two times will be more marked if they are plotted against the pH values of



Graph showing effect of pH on times of gelation and complete opacity

resulting mixtures. Hence, it is possible to reverse the order of two processes by simply changing the pH of the reaction medium. The pH at which the order of these two processes can be reversed depends on the relative values of times of gelation and complete opacity of the original serum. The difference in the rate of variations of these two times points strongly to the suggestion that gel-formation and development of opacity are caused by two different proteins, but the coincidence of the pH value of the minimum times of gel-formation and opacity suggests otherwise. In the preceding section, however, we have shown that with formalin opacity is more marked on the alkaline side when it is added to a mixture of 0.05 cc of kala-azar serum and 1 cc of buffers of different pH values. What is the reason for this apparently paradoxical behaviour of formalin under these conditions? On the acid side, opacity is more marked when there is a gel and is less marked in its absence. The obvious reason is that during the process of gelation a large quantity of water is absorbed and, therefore, the process of gel-formation helps the precipitation of euglobulin by taking up water. From this point of view, the quicker the rate of gel-formation the quicker also will be the rate of precipitation of euglobulin and, therefore, at the pH value of minimum time of gelation, there will exist also the minimum time of complete opacity. On the acid side of the pH of minimum time, gel-formation is retarded to a greater extent than the process of development of opacity, at a lower pH value the rate of development of opacity should be similarly retarded due to the slower rate of absorption of water by the gel, but the pH being

Our thanks are due to Lieut-Col H H King, I M C, the Director of the Institute, for suggestions and guidance during the course of the experiments

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## APPENDIX

*The histories of the 32 cases of elephantiasis (Group A in Table I) giving briefly the results obtained*

Note —+++ = marked adhesion in all the mf seen

++ = " " " most but absent in a few

+ = moderate adhesion in many but absent in a few

— = absence of adhesion

No	Name, sex and age	History and clinical signs	History of attacks of fever	Maximum duration of disease	Adhesion	REMARKS
1	A B, f, 38	Elephantiasis both legs, fullness inner side of both elbow joints where skin is thickened, right epitroch +, inguinal?	Attacks every six months	12 years	+++	
2	J, f, 30	Both legs affected, but not feet, both epitroch +, groin?	Once a month	2 years	+++	Clumping of mf seen
3	G, m, 18	Left leg and left arm affected, inguinal and femoral glands ++, left epitroch +++, hydrocele +	One attack only, nine months ago	1 year	+++	"
4	B J, f, 18	Right leg affected, right groin glands +, epitroch —	Four attacks in a year, last attack six months ago	1½ years	+++	
5	S A, m, 33	Slight fullness of ankle and left foot.	Frequent	1 year	++	Clumping of mf seen

a gel within an hour when the serum is diluted with an equal volume of saline. The greater the time of gel-formation of the original serum, the longer also will be the time of gel-formation of the diluted serum. In fact, the ratio in which the time of gel-formation of the diluted serum will increase also depends on the time of gel-formation of the original serum. If the latter be under 1 minute, the ratio will be smaller than in a case where the time of gel-formation of the original serum is 16 to 20 minutes. When other diluents are used, the effect of these will in general be determined by the change of pH of the reaction medium, and the decrease of the concentration of the particular protein in the serum. When the diluents react chemically with either formalin or impurities present in it, or with any of the constituents of the serum, it is difficult to predict what will happen.

#### IV IS BUFFER ACTION A FACTOR IN FORMALIN REACTION? EXPLANATION OF THE ALDHYDE TEST FOR KALA-AZAR

The reason for the difference in the times of gelation and complete opacity of different kala-azar sera can be explained now. The concentrations of the proteins which are responsible for this gel-formation and opacity will of course differ in different samples of blood sera and, therefore, opacity might precede gelation or gelation may appear before development of complete opacity. We have shown above that the concentration of the protein has a great effect on the times of gel-formation. When the concentrations of proteins in a sample of serum are sufficiently high, both the times of gelation and complete opacity are much shorter. Moreover, the change of pH caused by formalin will also have to be taken into account. We know that when 0.05 cc of formalin is added to 1 cc of phosphate buffer of pH 7.4, the pH of the resultant mixture is somewhere between 7.4 and 7.3 as determined by the colorimetric method. When, however, 0.1 cc of formalin is added, the pH of the resultant mixture is somewhere between 7.2 and 7.3. The results are true if we assume that formalin does not displace the equilibrium between the dissociated and undissociated portions of the indicator at a particular pH. Most probably it does not. The buffering action of a phosphate buffer is of course much higher than that of normal serum and therefore, still higher than that of serum from the blood of kala-azar patients. Therefore, when 0.05 cc of formalin is added to 1 cc of kala-azar serum, the pH of the serum is certainly below 7.3, we had to argue indirectly since when 0.05 cc of formalin is added to 1 cc of kala-azar serum, it sets within a short time and we cannot measure the pH of a white opaque jelly. It is thus obvious that formalin does change the pH of the serum and hence regulates the appearance of gel and opacity. The higher the concentrations of the proteins the lower is also their buffer action and, therefore, both concentrations of the proteins and their buffer action tend to make the times of gelation and complete opacity shorter. The lowering of pH on the alkaline side will diminish the time of complete opacity much more than

APPENDIX—*contd*

No	Name, sex and age	History and clinical signs	History of attacks of fever	Maximum duration of disease	Adhesion	REMARKS
16	M M, m, 40	Both legs, penis and scrotum affected, both groins and both epitroch +	Frequent	15 years	++	
17	P M, m, 33	Both legs affected, both groin glands +, right epitroch +	"	4 years	++	
18	K., m, 22	Right leg affected, hydrocele left, both groin glands +, epitroch. both +	"	6 years	++	
19	R., m, 17	Both legs affected, hydrocele both sides, both femoral glands +	Very frequent	1½ years	++	
20	G., m, 27	Both legs, right arm and scrotum affected, right epitroch and axillary +	"	7 years	+	
21	P., m, 12	Right leg affected, both groin glands ++	"	6 months	—	
22	P., f, 30	Left ankle affected, both epitroch +	"	1 year	+++	
23	D., m, 33	Both legs, scrotum and right arm +, both epitrochs and right groin glands +	"	15 years	+++	
24	S M, m, 32	Right leg affected, right epitroch and inguinal +, right hydrocele	Once in three months	3 years	+++	
25	K., m, 23	Right leg affected, both groins +	Very frequent	1½ years	—	
26	M N, m, 30	Left leg and right forearm affected, left hydrocele, left epitroch and groin glands affected	"	7 years	++	

A case which is nine months old has, as its time of gelation, only 50 seconds, whereas a case which is one year old has, as its time of gelation, 18 minutes. If we do not doubt the truth of the history of the case as given by the patient, these experiments are sufficient to prove that there is no hard and fast relation between the duration of the disease and the time of gelation. This point requires further investigation which is being done. From the observations we have made up to now, we find that time of gelation depends on the acuteness of the disease.

### GENERAL DISCUSSION

In this concluding section, we shall sum up the results of our work with a short discussion on the subject. We have shown that there is a particular pH at which the minimum times of gelation and complete opacity occur. This pH value shows also a maximum of opalescence where 0.05 c.c. of kala-azar serum and 0.05 c.c. formalin and 1 c.c. of buffer of different pH values are mixed. The rate of decrease of gelation is higher than the rate of decrease of opacity on the acid side. These facts of course suggest that gel-formation and development of opacity are caused by two different proteins, but in view of the complicated system with which we are working, there may be other unknown factors which bring about these phenomena. The possibility that formalin, reacting with euglobulin, might change its nature cannot be excluded, and this suggestion might explain the facts observed equally well. At the present state of our knowledge in the case of pure proteins—not to speak of such complicated systems—it is quite impossible to decide between these two assumptions. But whatever may be the theory which will explain these experimental observations, these facts give us a clearer view of the processes of gel-formation and development of opacity in kala-azar serum. Further investigations are necessary to isolate and identify the protein, if gel-formation is really due to a different protein than euglobulin. Whether this protein is present in normal and other pathological sera is also a question for investigation. Moreover, there may exist a specific effect of the phosphate buffers on gelation and opacity.

Further work is being done on these lines which we hope to publish shortly.

### SUMMARY AND CONCLUSIONS

The main conclusions which can be drawn from our experiments are the following —

- (1) For a particular kala-azar serum, there is a definite pH at which the times of gelation and complete opacity with formalin are minimum. In some cases they occur at the pH value 6.94 and in others at the pH value 7.27.
- (2) The rate of decrease of opacity is much greater than the rate of decrease of gelation on the alkaline side, whereas the rate of decrease of gelation is greater than the rate of decrease of opacity on the acid side.
- (3) By dilution with an equal volume of conductivity water or physiological saline solution, etc., the gel-formation is retarded.

A NEW FILARID IN *CALOTES VERSICOLOR*—  
*CONISPICULUM GUINDIENSIS*, N G, N SP

BY

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EARLY in 1927 we undertook a study of the filarial infection in *Calotes versicolor*, the common 'bloodsucker,' with a view to see if it would prove suitable for experimental purposes. A large percentage (19 per cent) of these animals caught in the vicinity of the Institute are found to be infected with a filaria. We have been able to find in literature only one record of a filarial infection of this lizard. O von Linstow (1906) gives a brief description of the worm under the name *Filaria flavescens* (Castellani and Willy, 1904). The filaria now being described differs from it in some important respects, e.g., the length of the worm and the position of the genital opening in the female.

The worm belongs to the sub-family MICROPLEURINÆ and possesses the following distinctive features of the sub-family — 'Mouth simple without a chitinous peribuccal ring or epaulette-like structures, without chitinous tridents on each side of the anterior end of the œsophagus, cuticle smooth, spicules equal, vulva near the middle of the body' (Yorke and Maplestone, 1926). The only genus described under this sub-family is *Micropleura* (Linstow, 1906). Our filarid differs from this genus in the following respects —

- (1) Absence of lateral and submedian head papillæ
- (2) Absence of caudal alæ in the male
- (3) Spicules short and stout, and not slender and tapering to fine points

The above points of difference are so fundamental that we feel justified in erecting a new genus in the sub-family MICROPLEURINÆ

# STUDIES IN PHYSICAL PROPERTIES OF DIFFERENT BLOOD SERA

## Part III. VISCOSITY

BY

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THE present work was undertaken with a view to see whether there exists any marked variation in the viscosity of normal and pathological sera. Since higher animal life depends on the proper circulation of blood through a complex network of tubes of various dimensions, arteries, veins, arterioles, and capillaries, it is obvious that the viscosity of blood and serum must be a matter of some importance. The circulation of the blood in the body is brought about by the pumping action of the heart and it will, therefore, be clear that for a given strength of heart-beat and for a given rate of beat, the pressure in arteries will depend on the so-called 'peripheral resistance'.

Although according to Albutt (1911) much work has been done on the viscosity determination of the normal blood and serum, comparatively less attention has been paid to determinations of different pathological sera. The most important of such determinations were done by Naegali (1919) and by Bircher and his co-workers (1921). Naegali thinks that a simultaneous examination of the viscosity and refractivity of a sample of serum reveals the relative proportion of albumin and globulin although neither of these properties singly gives any idea of this proportion. The methods of determination of the constituent proteins, as developed by Reiss (1913) and later on by Robertson based on measurements of refractivity alone, are open to objection. Bircher and his co-workers (*loc cit*) say that a large series of experiments have shown that there are discrepancies, which can only be explained by the differences in



Average distance of the anus from posterior end, 0.41 mm

The lumen of the intestinal tube is usually filled with a brownish material

*Reproductive system*—The major portion of the body cavity is occupied by the two tubular uterine tubes, which are filled with eggs in various stages of development. The ovaries and oviducts were not seen, as they were masked by the uterine coils. The two uterine tubes fuse to form a vagina which opens about the middle of the body, at the summit of a papilla. The worm is ovoviviparous. Both fully developed eggs enclosing coiled up microfilariae and free embryos were observed being ejected through the vaginal orifice.

*Nervous System*—A nerve ring round the oesophagus just before it expands into the bulbous portion.

Average distance of nerve ring from anterior end, 0.27 mm

II—Male—Much smaller than the female and distinguished from it by its size and by its coiled, loose, spiral tail, sharply curved ventrally.

Average length, 0.28 mm

Average breadth anterior end, 0.24 mm

„ „ middle, 0.3 mm

At the tip of the tail two clear, rounded, fairly big bosses are seen, and between these and the anus are two rows of papillae—usually 7 pairs—the papillae diminishing in size from the anus downwards.

*Alimentary Canal*—Resembles that of the female.

*Oesophagus*—Average length of anterior narrow portion, 0.3 mm

„ „ „ posterior broader portion, 0.74 mm

„ „ „ the entire oesophagus, 1.04 mm

*Intestine*—As in the female the tubular intestine gets narrowed at the anal region and opens out along with the genital opening.

*Reproductive System*—A long, coiled tubular testis, running the entire length of the body, leads to a vas deferens which enlarges in size before opening at the genital orifice. Here are two short conical spicules, almost equal in size and similar in shape. The spicules have a broad base and a tapering extremity which just protrudes from the genital opening (Plate LXXIX, fig. 1). As in the female, this opening is situated in the middle of a papilla.

Average distance of genital opening from posterior end

0.21 mm

Average length of spicule (1)

0.132 mm

„ „ „ (2)

0.14 mm

Average breadth of spicule (1) head

0.051 mm

„ „ „ tip

0.009 mm

„ „ „ (2) head

0.054 mm

„ „ „ tip

0.009 mm

readings was taken, the viscosimeter was cleansed thoroughly with sulphuric-chromic acid mixture, washed with conductivity water, and then dried in an air-oven. When one type of serum is used, the viscosimeter was not cleansed for subsequent determinations of different samples on the same day. The results are given in the following table —

TABLE  
*Relative viscosity of different sera at 25°C.*

No	Normal	Leprosy	Syphilis	Kala-azar
1	1.40	2.42	1.90	2.0
2	1.61	1.62	1.68	2.28
3	1.40	1.46	1.60	2.28
4	1.62	1.61	1.72	2.28
5	1.56	1.61	1.62	2.17
6	1.50	2.05	1.83	2.38
7	1.61	2.16	1.72	2.61
8	1.50	1.61	1.61	2.93
9	1.50	1.50	1.73	2.38
10	1.58	1.73	1.72	2.72
11	1.55	1.73	1.62	2.29
12	1.45	1.38	1.73	2.11
13	1.50	1.73	1.61	1.65
14	1.49	1.5	1.83	2.50
15	1.63	1.38	1.89	1.9
16	1.57	1.61	1.72	2.40
17	1.48	1.48	1.83	2.67
18	1.60	1.50	1.95	2.67
19	1.55	1.61	1.83	2.2
20	1.64	1.45	1.72	3.2
21	1.45	1.50	1.95	2.22
22	1.58	1.50	1.62	2.10
23	1.42	1.45	1.72	2.30
24	1.55	1.48	1.83	2.45
25	1.60	1.61	1.79	2.67

*Cephalic Extremity*—is blunt and rounded. The body cells begin a little behind the anterior extremity leaving a clear space in front—the 'cephalic space,' which measures 5 microns.

*Nerve Space*—A narrow transverse break in the column of cells at a distance of 24  $\mu$  to 28  $\mu$  from the cephalic extremity.

*Excretory Pore and Cell*—The excretory pore is seen as a clear space without granules at a distance of 40  $\mu$  from the anterior end. Just posterior to the pore and generally at a distance of 2  $\mu$  or 3  $\mu$  is a 'big' granule which is probably the excretory cell. Under the dark ground in fresh specimens this is seen as a brownish shadow in the column of refractile cells.

*Anal Spot*—The rudimentary anal opening is a clear space, 76 $\mu$  to 84  $\mu$  from the anterior end. The body cells are seen to extend beyond the anal pore.

*Body Cells*—The nuclei are arranged in rows of 4 and 5 and do not fill up the whole body of the embryo, there being a clear margin between the cuticle and the nuclear column. The nuclei are clear and distinct and can be counted easily in specimens stained with Meyer's Hæmalum or Azure II.

No cephalic spine can be made out. A funnel-shaped invagination is noticed at the cephalic end when the embryo retracts its head.

Our thanks are due to Dr P. A. Maplestone, D.S.O., M.D., for examining our specimens and to Lieut-Col H. H. King, I.M.S., the Director of the Institute, for advice and guidance during the course of the work.

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#### EXPLANATION OF PLATE LXXVIII

- Fig 1 Anterior end of female, *C. guindiensis*, showing nerve-ring and oesophagus  
 „ 2 Posterior end of female  
 „ 3 Anterior end of male  
 „ 4 Posterior end of male, showing spicule  
 „ 5 Tail-end of female, showing the anus and caudal papillæ (higher magnification)  
 „ 6 Tail-end of male, showing spicules (same magnification)

associated with different proteins (say  $n$ ) comes out as follows from the experiments of H. Chick mentioned in the foot-note

	$n$
Euglobulin	2.2
Pseudoglobulin	1.5

From these considerations as well as from arguments of Heyder and Rohrer (*loc cit*) it is clear that when the relative viscosity of a serum is high, we have to assume that it contains higher percentage of protein. The serum in which there is relatively more of euglobulin should show the highest viscosity. In fact the sera from the blood of kala-azar patients exhibit relatively an abnormally high value for the relative viscosity. The values for viscosity of kala-azar serum lie between 2.0 and 2.9. Such values are to be considered exceptionally high. In a previous paper the authors (1928) have shown that the surface tensions of tubercular and kala-azar sera exhibit the lowest value. It was also suggested that the lowering of surface tension in both the cases was due to a relative increase in euglobulin. Determinations of viscosity of tubercular sera have been carried out by Bircher (*loc cit*). According to him 'in the progressive stage of the disease, the viscosity is greatly increased. Almost one half of all the sera of this group have a viscosity between 2.0 and 2.10. A viscosity of 2.3, 2.4 and even of 2.6 as found in several instances, is to be considered exceptionally high. Such degrees of viscosity were not observed in the great number of determinations made during the past year in other diseases. As expected, the globulin is high, specially in cases of progressive tuberculosis.' Correlating these observations with our data on surface tension and formol-gel test published in our previous papers, we might remark that the lowering of surface tension, observed in advanced cases of tuberculosis, is due to a relative increase of euglobulin in these sera. This also explains why we get a positive antimony test in these cases. Our former statement 'we shall not be far from the truth if we say that the lowering of surface tension in these cases of tubercular sera is caused by the relatively greater concentration of euglobulin' finds additional support from the facts mentioned above.

The viscosity of syphilitic sera varies from 1.6 to 1.9. In syphilitic sera, pseudoglobulin increases, but it is not so effective in increasing the viscosity as euglobulin as stated above and it is not surprising that in all these sera, which are cent per cent Wassermann positive, the values for viscosity are not higher than those obtained either with tubercular or kala-azar sera. Bircher and his co-workers found (*loc cit*) that 'the viscosity of serum, based on 174 determinations in persons known to be syphilitic, was usually found to lie between 1.70 and 1.90. The lowest rate observed was 1.60 and the highest 2.05. The average was 1.79.' Comparing these values after correcting for temperature, we see that our results agree quite well with the results obtained by Bircher and others.

PLATE LXXIX

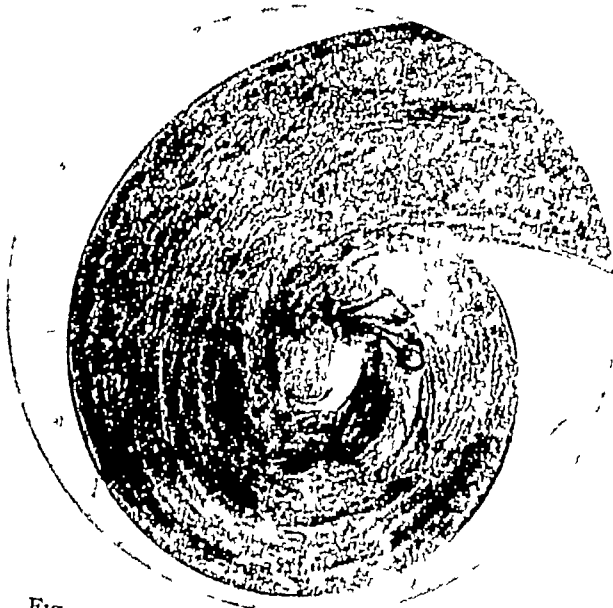


Fig 1—*C. gundensis* Posterior end of male  
showing spicules (low power)

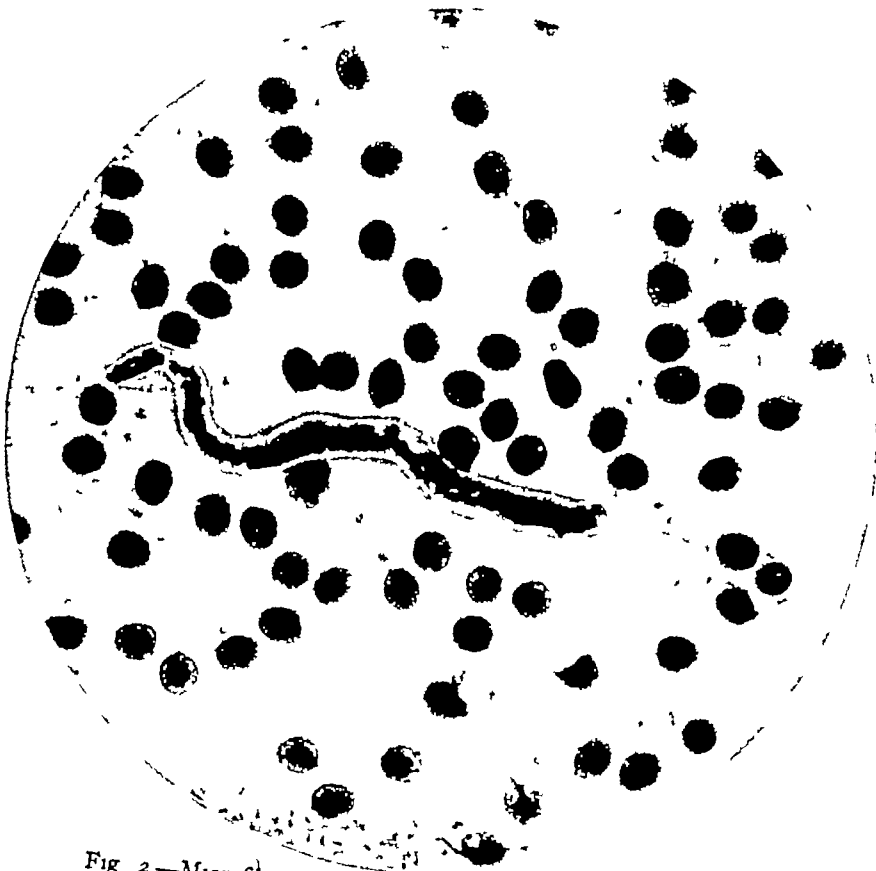


Fig 2—Microfilaria of *C. gundensis* showing sheath

- OCHLECKER (1910) *Berl Klin Woch*, XLVII, 578
- SIMON (1911) *Beitr Z Klin Chir*, LXXII, 125—151
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base, length of spicules,  $84\ \mu$ , maximum breadth,  $18\ \mu$ , alæ absent A row of three minute papillæ present on either side beyond the anus

*Female* Average length,  $24.5\ \text{mm}$

Maximum diameter,  $0.33\ \text{mm}$

Two to three times longer than male, anterior end rounded and devoid of papillæ, mouth simple leading to a stout œsophagus  $700\ \mu$  in length, œsophagus not divided into two parts but widens out posteriorly, nerve ring  $138\ \mu$  from anterior end, genital opening in the œsophageal region,  $310\ \mu$  from cephalic extremity, tail short, anus  $400\ \mu$  from caudal end, ovo-viviparous, embryos were observed emerging through the genital orifice and measured  $132\ \mu$  long and  $3.8\ \mu$  broad, average diameter of eggs in the vulva  $10\ \mu$ .

*Habitat* Heart and probably the larger blood vessels

*Microfilaria* Peripheral blood of birds Enclosed in a loose sheath, exhibits active wriggling movements without change of position

Average length,  $152.1\ \mu$

Average breadth,  $3.3\ \mu$

Blunt anterior end and a tapering posterior end, nuclei arranged in solid masses, cephalic space wedge-shaped in appearance The microfilariae show a well marked nocturnal periodicity

TABLE I

*Anatomical measurements of microfilariae in microns (Leishman stain)*  
All measurements from the anterior end

	1	2	3	4	5	6	Average	Percentage of total length
Length	163.8	151.0	149.0	138.0	155.0	156.0	152.1	
Breadth	2.8	2.8	3.1	3.5	3.5	4.0	3.3	2.2
Break 1 (Cephalic)	2.8	2.8	2.8	2.9	3.1	2.8	2.9	1.9
Break 2 (Nerve ring)	43.7	38.2	38.2	36.3	41.9	42.3	40.1	26.3
Break 3 (Excretory pore)	61.9	54.6	54.6	51.0	58.2	59.3	56.6	37.2
Break 4	111.0	100.1	96.0	94.2	103.7	103.9	101.5	66.7
Break 5	127.4	132.8	114.6			138.2	128.3	84.4
Break 6 (Anal spot)	147.4	..	132.9	126.0	139.2	.	136.4	89.7
Break 7	160.1		.	135.6	152.0	..	149.2	98.1

PLATE LXXVI

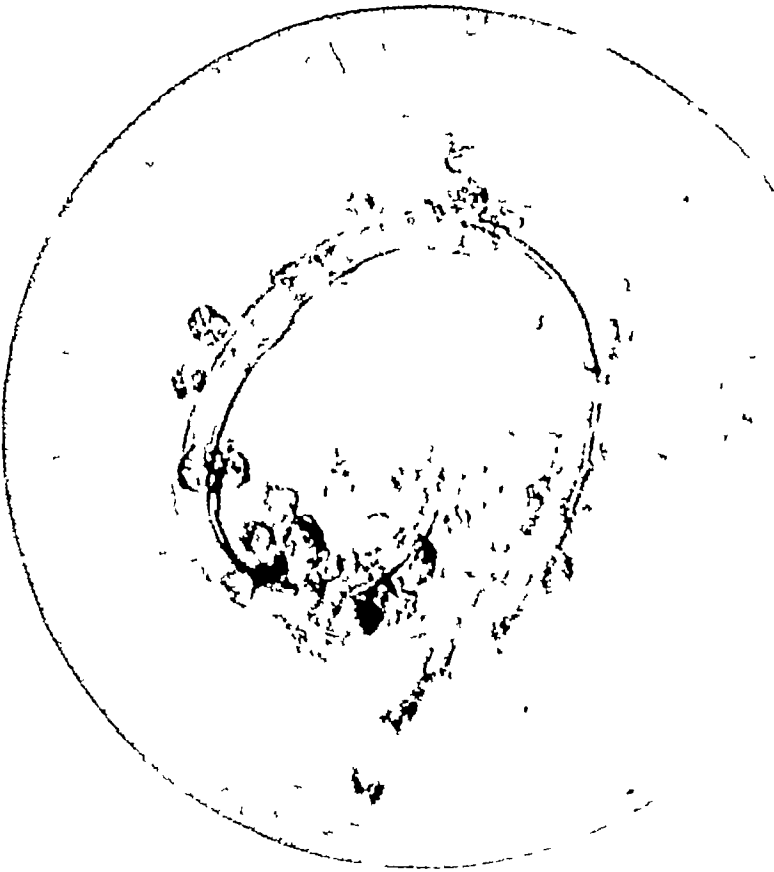


Fig 1—*Microfilaria bancrofti* showing adhesion of leucocytes, early stage One hour after incubation

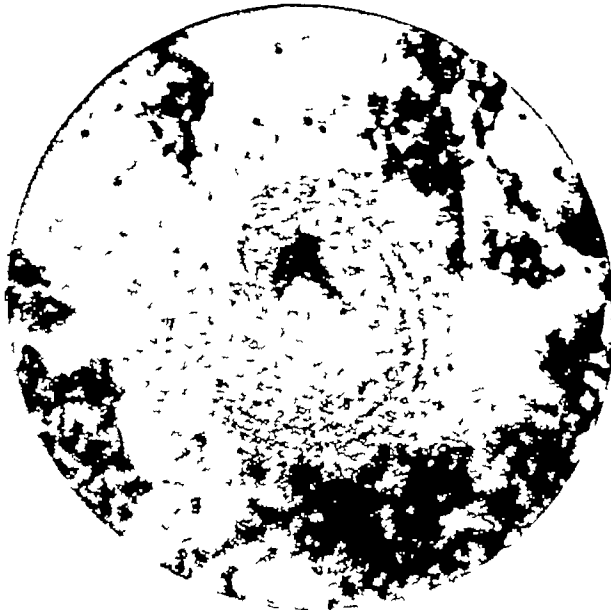


Fig 2—Marked adhesion of leucocytes.  
16 hours after incubation



described above belonged to this species was proved by the close resemblance of the embryos extruded by the parent worm and those found in the blood

That the description given in this paper of the two sexes relates to the same species is clear for the following reasons —

1 All the worms were obtained together in the right ventricle of the heart in the blood clots

2 Even though the crow harboured two kinds of microfilariae, as was the case with Chandler, the actual measurements of the embryos extruded by the parent worm were taken and found to correspond to the longer variety of the microfilaria

It is therefore probable that Chandler's description of the female of *C bosei* refers to some other species and the female described in this paper is the correct description of the worm in question

#### CONCLUSION

1 The female of *Chandlerella bosei* and its microfilaria are described The female worm described by Chandler is evidently a different species

2 The microfilaria of *C bosei* shows a well marked nocturnal periodicity

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'New Filariæ from Indian Birds' *Parasitology*,  
Vol XVI, p 398

at room temperature overnight. Strict aseptic precautions are necessary, for bacterial contamination and consequent hæmolysis prevent the occurrence of the reaction. The same volume of the microfilarial suspension in a glass vial serves as a control.

It is necessary here to point out that the concentration of citrate in the mixture plays an important role. The microfilarial suspension is best used in a dilution of 4 to 1 with 2 per cent citrated saline solution as already noted. If the concentration is halved, clotting takes place on the addition of sera. On the other hand, we have observed that too high a concentration inhibits the phenomenon.

The mixtures are conveniently examined next morning, i.e., after 12 to 14 hours' incubation, though a shorter incubation of one or two hours is sufficient. Each tube is rolled between the fingers to obtain an even distribution of microfilariae in the mixture. A drop is transferred to a slide and examined under a coverslip with the low power of the microscope. If a fairly rich emulsion is used, microfilariae are easily found. They sometimes, however, adhere together in small or big clumps. In such cases many preparations may have to be examined before a single specimen is encountered.

In a positive reaction, as is met with in an elephantiasis serum, the leucocytes are found attached to the microfilariae along their entire surface (*see* Plate LXXVI, figs 1 and 2). The microfilariae become sluggish and in some cases even perish. In the majority of cases they are seen singly, but large and small clumps with adherent leucocytes also occur. Normal sera do not show the reaction. Here the microfilariae are more active and are often found in clumps which are as a rule bigger than those met with above (*see* Plate LXXVII). Dead microfilariae are rarely seen and adhesion of leucocytes to them does not take place. It is quite usual to see leucocytes caught amongst the clumping microfilariae, but these are not adherent and the appearance is quite unlike that met with in a positive reaction.

Once the adhesion takes place, the leucocytes continue to adhere even after 72 to 96 hours, though they may be dead and disintegrating. Blood platelets probably adhere, but they cannot be distinguished in the mass of adhering leucocytes. Red cells do not adhere and iso-agglutinins do not interfere with the reaction.

So far 62 sera have been examined. They are grouped under the following heads —

- A Those obtained from well marked cases of elephantiasis with *active* disease. None had microfilariae in their night blood.
- B Sera from persons having microfilariae in their blood without any clinical lesions.
- C Sera from persons who were apparently healthy at the time of examination, but who gave histories of transient filarial infection many years ago. Microfilariae were not found in their blood at any time.

7.11.1

1

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It is interesting to speculate if the positive reaction in the three sera in D and E is due to repeated attacks of infection without the production of any clinical manifestation.

We have not so far examined sera from cases of elephantiasis with microfilariæ. From our experience of the filarial survey of Saidapet, whence all our elephantiasis sera were obtained, we found such cases to be very few. It would be interesting to know how such sera would react to this phenomenon.

A few facts with regard to this reaction will be briefly stated —

1 The anti-body is thermo-stable, withstands heat at 56°C for half an hour.

2 The phenomenon is specific for *M. bancrofti*. Action on the microfilariæ of the crow (*M. Chandlerella bosei*) sera have no effect. (The lizard (*M. Conspiculum guindensis*)).

3 The reaction is seen equally well when the mixtures are incubated in atmospheres of CO<sub>2</sub>, H<sub>2</sub>, or air.

4 The adhesion is not noted with yeast cells and dead *B. coli*.

The nature of the anti-body concerned is being investigated and will form the subject of another communication. That it is of the nature of an opsonin does not seem unlikely from the fact that in the positive reaction microfilariæ become very sluggish and soon die. The leucocytes do not serve as mere 'scavengers' for removing dead or dying microfilariæ, for the reaction begins to appear within an hour, i.e., before the activity of the microfilariæ has diminished to any extent, the attachment being firm enough to prevent dislodgment of cells during the movements of the microfilariæ.

It is also found that when the activity of microfilariæ in control sera has ceased after two or three days, the addition of fresh leucocytes does not result in the production of the phenomenon. A similar phenomenon was observed by Levaditi and Mutermilch (1910) in trypanosomiasis. These authors note two phases of the reaction, the first being the attachment of leucocytes to the sensitized trypanosomes and the second that of proper englobement and destruction of the trypanosomes by the leucocytes. The first they describe as a physico-chemical reaction analogous to agglutination and the other active phagocytosis.

Davis and Brown (1927), who have reviewed the previous literature and named this reaction the 'adhesion phenomenon,' have further shown that it can be obtained even with dead bacterial emulsions or any inert particulate matter. As has been already noted, we have not observed such adhesion in filariasis with bacteria and yeast cells.

### CONCLUSIONS

The phenomenon of adhesion of leucocytes found in protozoal infections is shown to occur also with microfilariæ in elephantiasis, a metazoan infection. This reaction may serve as an additional test in the diagnosis of filariasis, but its chief importance appears to be the light it throws on the mechanism of immunity in filarial disease.

that the Indian troops in Macedonia included Gurkhas, Garowalis, Rajputs, Jats, Kumaons and Labour Transport Corps composed chiefly of low caste people from different parts in India, it seems fairly certain that they were dealing with a heterogenous population in which Mongolians, Indo-Aryans and Dravidians were represented

Their theory is based on a complete misconception of India and Indians. For example, they say, 'The Indians who are looked on as anthropologically nearest to Europeans show the greatest difference from them in their blood properties'. A slight acquaintance with India would have made it apparent to them that only the inhabitants of the Punjab and the North-West Frontier Province, generally speaking, can be considered as anthropologically near to Europeans, and these are not the early inhabitants of India but foreigners who invaded the country in comparatively modern times. It is curious that this lack of knowledge of the great diversity of races constituting the population of India has not been pointed out by writers who have supported or criticized the Hirschfelds' theory.

Ottenburg (2 and 3) who collected the figures of many authors from 1919 to 1924 classified the peoples of the world into six types on the percentages of the four blood groups retaining the 'biochemical race index' of the Hirschfelds but he obscures the question still further, so far as India is concerned, by confusing race with religion, since he described Hirschfelds' 'Indians' as 'Hindus'.

Snyder (5 and 6) pointed out that the 'biochemical race index' is not mathematically adequate and formulated a classification of races on the frequencies of the hereditary factors A, B and O, employing Bernstein's hypothesis that blood groups are inherited as three multiple allelomorphs, A and B being dominant to the recessive O, instead of on the basis of two independent pairs of Mendelian factors, A being dominant to a and B to b, as supposed by Ottenburg (4). According to Snyder (5 and 6)  $p = 1 - \sqrt{\text{group O} + \text{group B}}$ ,  $q = 1 - \sqrt{\text{group O} + \text{group A}}$ ,  $r = \sqrt{\text{group O}}$  where p represents the frequency of the factor A, q of the factor B and r of the factor O.

In view of the fact that no observations on blood groups in Indian races have been published, the writers think that it would be of interest to present in this paper a brief report of the results obtained in their study of this aspect of the question.

According to Risley in the Census Report for India, 1901, (7) it is possible to divide the peoples of India into six distinct types according to certain anthropological characteristics. We have been able to examine individuals representing three of these types, the Turko-Iranian, Indo-Aryan and Dravidian. The Turko-Iranian of our series comprise Indian troops stationed at Quetta [studied by one of us (R H M) five years ago] as well as a certain number of patients attending the Pasteur Institute, Kasauli. The Indo-Aryans were selected from the Pasteur Institute patients and are all natives of the Punjab. The figures for the Dravidian group are the result of an inquiry conducted by one of us (M N L) under a grant from the Indian Research Fund Association. The last-named

## APPENDIX—contd

No	Name, sex and age	History and clinical signs	History of attacks of fever	Maximum duration of disease	Adhesion	REMARKS
6	B, m, 36	Right orchitis and funiculitis, slight fullness of left ankle, recent, hydrocele both sides, skin thickened, left epitroch +	Every four months till 1920	12 years	+++	
7	M, m, 35	Right forearm affected, right epitroch +	Fever	6 years	—	Clumping of mf seen
8	G I, m, 28	Fullness of right ankle with thickened skin, right hydrocele, both epitroch +, femorals and inguinals —	No history of fever	3 years	—	
9	P, f 15	Swelling left foot, right epitroch +, groin?	Frequent attacks	4 months	+++	
10	K., f, 11	Right leg affected both epitroch +, groin?	„	3 months	+++	
11	A J, m, 46	Both legs elephantoid, groin glands +	Once in six months	10 years	++	Six out of 7 mf seen, showed adhesion
12	M, m, 25	Fullness right ankle, thickening of skin both shins, both epitroch +, groin +, double hydrocele	No history of fever	6 months	—	
13	S M I, m, 30	Left leg affected, left groin glands +	Had five attacks of fever	1 year	—	
14	M G, m, 22	Right thigh affected, right groin glands +, double hydrocele	Once a year	3 years	+	
15	B, m, 60	Both legs affected, scrotum removed for elephantiasis, right groin glands +	Once a month	25 years	++	Clumps

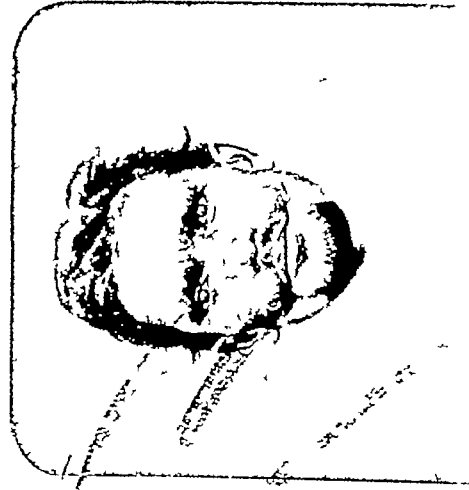


Fig 1

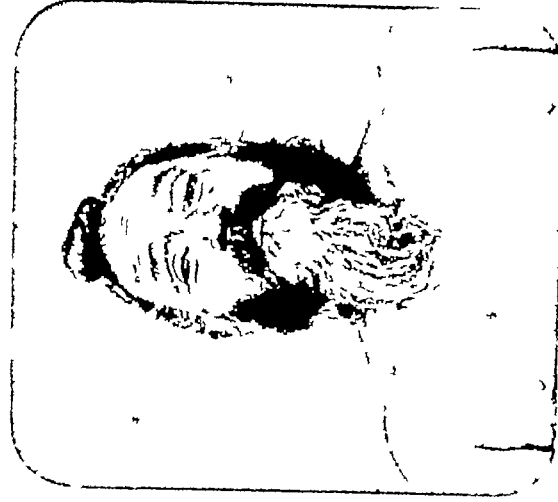


Fig 3



Fig 5



Fig 6

APPENDIX—*concl'd*

No	Name, sex and age	History and clinical signs	History of attacks of fever	Maximum duration of disease	Adhesion	REMARKS
27	S, m, 12	Right leg affected, left hydrocele, both groin glands +	Once a month	1 year	++	
28	B, f, 25	Left leg and right elbow affected, right epitroch +	Occasional, no definite history	5 years	—	Clumping of mf found
29	S M III, m, 31	Both legs, scrotum and right forearm affected, both groins and epitroch +	Once a month	17 years	+++	"
30	T K. S, m, 28	Left leg and right foot (recent), scrotum and penis affected, both groin glands +	Once in six months	8 years	+++	
31	D P, m, 20	Right leg affected, both groin gland +, double hydrocele +	"	5 years	+++	Clumping of mf seen
32	S, m, 26	Right leg and right forearm affected, right epitroch +, both groin glands +, double hydrocele	Once in 3 months	4 years	+	"



The test sera were selected after carrying out cross agglutination tests on a large number of persons, the results being confirmed by type sera obtained from the Lister Institute and Messrs Parke Davis & Co. They were always matched against our standard A and B cells immediately before using them for the tests proper, and no sera with a titre of less than 1 in 32 were employed.

The proportions of the blood-groups in the various races and castes examined are tabulated below —

	O	A	B	AB	Number of persons tested	p	q	r	Race index
Turko-Iranian	34.2	27.7	33.1	5.0	324	18.1	21.4	58.4	0.86
Pathan	29.3	31.3	33.3	6.1	150				
Baluch	47.2	24.3	24.3	4.2	74				
Hazaras	32.0	25.0	39.0	4.0	100				
Indo-Aryan	32.2	25.5	33.8	8.5	494	18.8	24.1	56.7	0.80
Rajputs	28.8	28.0	33.0	10.2	118				
Jats	33.2	24.5	35.5	6.8	277				
Khatri	33.3	25.3	30.3	11.1	99				
Dravidian	24.3	27.5	36.8	11.4	589	21.9	28.1	49.2	0.80
Hindus of various castes from United Provinces	30.2	24.5	37.2	8.1	2,357	17.9	26.1	54.9	0.71
'Indians' (Hirschfeld's figures)	31.3	19.0	41.2	8.5	1,000	14.9	29.1	55.9	0.56

As a control the bloods of 147 British soldiers stationed in Kasauli were tested. The frequencies of A, B and O in this group were as follows —  $p = 31.7$ ,  $q = 3.2$ ,  $r = 65.2$ . The figures agree fairly closely with those of the Hirschfelds' reported by Snyder (5 and 6), our value for  $p$  being higher and for  $q$  and  $r$  being lower than theirs.

#### Discussion

If the figures given in the column 'race index' be examined, it will be seen that there is little relation between the race index and anthropological characters, thus, for example, the Dravidians and the Indo-Aryans, two totally different types with regard to physical characteristics, manners, customs, language and history have the same race index.

If, however, the frequencies of  $A(p)$ ,  $B(q)$  and  $O(r)$  be calculated according to Snyder's formulæ, the relation between blood groups and anthropological characters becomes more evident. The Dravidian type has the highest frequency

Specimens with a description of the worms were sent to Dr P. A. Maplestone who was kind enough to examine them. He agrees with the description and suggests that the worm should go into the sub-family MICROPLEURINÆ, making a new genus to include it.

It is proposed to name the worm *Conspiculum gundiensis* n. g., n. sp., because of the characteristic conical appearance of the spicules under the low power of the microscope, and its presence in and around Gundy.

### *Conspiculum* n. g.

*Definition*—MICROPLEURINÆ, simple mouth without lateral or submedian head papillæ, cuticle smooth with fine longitudinal striations, œsophagus divided into a short narrow anterior, and a longer bulbous posterior portion. Male: absence of caudal alæ, short and stout equal spicules. Female: vulva in middle of body, two rows of caudal papillæ, ovo-viviparous, embryos in peripheral blood, no periodicity.

Type species—*Conspiculum gundiensis*, in *Calotes versicolor*. Type in the King Institute, Gundy, Madras.

Habitat—Loose connective tissue, chiefly mesentery, pelvic region, and along the trachea.

Description—(Plate LXXVIII, figs 1 to 6). Based on measurements of 6 of each sex.

I. Female—Long, stout and semi-transparent worm, 3 or 4 times as long as the male.

Average length, 95 mm

Average breadth, anterior end, 0.5 mm

Middle, 0.73 mm

Posterior end, 0.31 mm

Head round, tail blunt, head devoid of papillæ. A yellow pigment is sometimes to be seen in old and dead worms. Two rows of papillæ at the caudal end, about 7 on each side.

*Cuticle*—Smooth, with fine longitudinal striations—no bosses or elevations found on the body surface except two, one in the middle of the body—the vaginal opening, and the other at the anal region.

*Alimentary Canal*—Mouth simple with two inconspicuous lateral (?) obtuse flaps. No buccal cavity.

*Œsophagus*—Muscular, extends up to the anterior end and is divided into two portions (a) cylindrical short anterior portion, (b) a longer bulbous posterior portion.

Average length of (a) 0.33 mm

" " " (b) 0.9 mm

" " " œsophagus 1.23 mm

*Intestine*—Straight tube beginning immediately behind the œsophagus with a constriction and running through the length of the body. The tubular intestine gets narrowed towards the posterior end and opens out on an elevation.

5 No evidence is adduced to support the theory that agglutininogen A arose in Europe and B in India. It is at least as probable that both A and B arose in the Indo-African continent in the common ancestors of the anthropoids and man.

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- (5) SNYDER, L. H. (1926) *Amer Jour Physical Anthropol*, Vol IX, No 2, p 233
- (6) *Idem* (1927) *Arch Path and Lab Med*, Vol IV, p 215
- (7) RISLEY and GAIT'S Census report for India, 1901, Vol I
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C (1925)

*Nervous System*—Similar to that of the female

Average distance of nerve ring from anterior end 0.19 mm

### III—Microfilaria

#### *In unstained films*

Length 86 to 100  $\mu$  — average 98  $\mu$

Breadth 7 to 10  $\mu$  — average 9  $\mu$

#### *In stained films*

Breadth .. 86 to 114  $\mu$  — average 102  $\mu$

Length 5 to 10  $\mu$  — average 8  $\mu$

*Anatomical measurements of the microfilaria in stained films (Leishman stain)*  
(All measurements are in microns from the cephalic extremity)

Number	Total length	Cephalic space	Nerve ring	Excretory pore	Excretory cell	Genital cell	Anal pore	Last caudal cell
1	92	4	24	36	40	52	76	90
2	102	4	24	40	44	56	82	96
3	104	5	28	40	42	58	84	102
4	102	3	26	36	40	54	84	98
5	104	6	28	40	42	56	83	100
6	102	5	25	40	42	58	80	100
Average	101	5	26	39	42	56	82	98

*Movement*—Wriggling but not translatory movements

*Appearance*—Cylindrical in shape, rounded anteriorly, and tapering posteriorly

*Sheath*—Sheath present. It extends beyond the two extremities and is very well made out in specimens stained with acetic acid gentian violet (Plate LXXIX, fig 2). The microfilaria is seen alternately to advance and withdraw in the sheath, which envelopes the worm so loosely as to permit the doubling up of the worm inside. On two or three occasions it was even found to reverse its position, the head-end coming to occupy the tail-end of the sheath.

*Cuticle*—Smooth and transversely striated

A comparison of the results of crossing which should be obtained on the basis of each of the two hypotheses is given in Table I —

TABLE I

Crosses	Two independent pairs of factors	Three multiple allelomorphs
O × O	O	O
O × A	O, A	O, A
A × A	O, A	O, A
O × B	O, B	O, B
B × B	O, B	O, B
A × B	O, A, B, AB	O, A, B, AB
O × AB	O, A, B, AB	A, B
A × AB	O, A, B, AB	A, B, AB
B × AB	O, A, B, AB	A, B, AB
AB × AB	O, A, B, AB	A, B, AB

It is seen that the two hypotheses give the same results except in the offspring of crosses in which one of the parents is AB. In order to throw some light on this question I examined the parents and children of 49 families using the technique previously described(4). The results of 46 families are shown in a condensed form in Table II —

TABLE II

Crosses	Number of families examined	Offspring
O × O	4	O
O × A	8	O, A
O × B	9	O, B
O × AB	2	A, B
A × A	6	O, A
A × B	9	O, B, AB
A × AB	1	A
B × B	5	O, B
B × AB	2	B

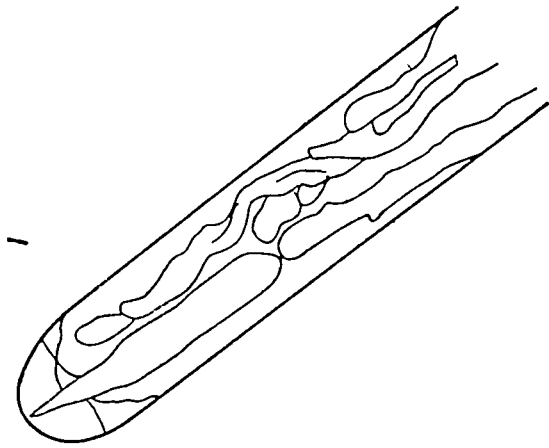
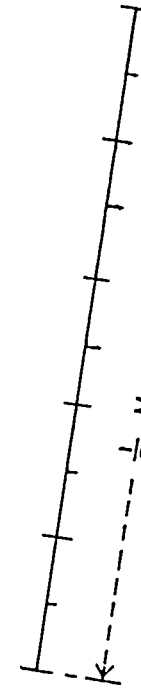
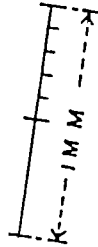
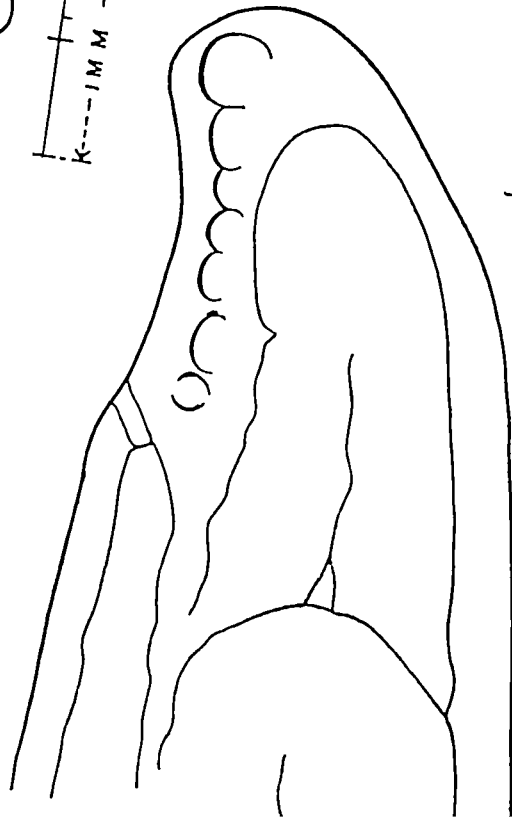
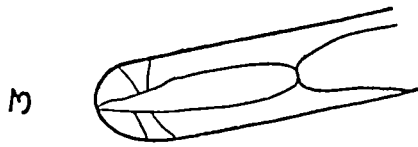
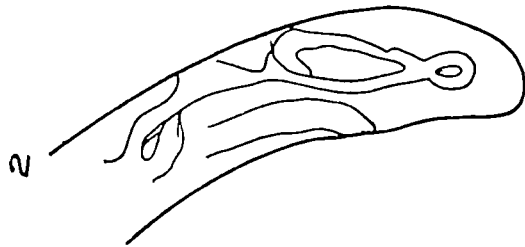


PLATE LXXVIII



6

5

further examination proved not to be so. The case described (family No 20) is an interesting example of polyandry of the fraternal type the existence of which was brought to light by blood grouping tests.

I have to thank Major R. H. Malone, I.M.S., for his advice in carrying out this work.

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- |  |   |
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| (4) MALONE, R. H. and LAHIRI, M. N. (1929) | <i>Ind Jour Med Res</i> , Vol XVI, No 4, pp 963—968 |
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A NOTE ON *CHANDLERELLA BOSEI*, YORKE AND  
MAPLESTONE, 1926

BY

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[Received for publication, October 15, 1928]

IN the course of a study on filariasis, we had occasion to examine a number of crows for the presence of microfilariae in the blood. Though the incidence of infection amongst the crows is high, adult worms were not easily obtainable either from the sinuses or the heart, their normal habitat. Two species of worms were obtained—one belonging to the genus *Diplotriana*, and the other *Chandlerella bosei*, originally described by Chandler (1924) as occurring in the bird *Dissemurus paradiseus*. In one crow, which showed a heavy infection with microfilariae, 13 adult worms, 4 males and 9 females, of *C. bosei* were found in the right ventricle. The female worm we found differs in some respects from the one described by Chandler, and as Chandler himself expresses a doubt whether the male and female described by him belonged to the same species, a short description of this worm seems to us necessary.

The following is a brief description of the worm based on 4 male and 9 female specimens —

*Male* Average length, 11.5 mm

Maximum breadth, 1.45 mm

Rounded anterior end, simple mouth without lips, no papilla at the cephalic extremity, smooth cuticle, posterior end spirally coiled with the tip bluntly rounded, spicules equal, tapering gradually with a slight constriction towards the



TABLE I

Date	Reaction pH	Sp Gr	Albumin per cent	Urea per cent	Fat per cent	Fibrin clot per cent
<i>Case No 1 (before breakfast urine)</i>						
16-9-27	7.5	1015	0.6	0.8	0.18	—
19-9-27	7.5	1013	0.5	0.7		+
20-9-27	8.0	1009	0.3	0.6		—
21-9-27	7.5	1010	0.3	0.5	0.67	—
22-9-27	8.0	1010	0.4	0.7	0.75	—
23-9-27	7.5	1009	0.3		1.1	—
<i>Case No 1 (three hours after the mid-day meal)</i>						
16-9-27	7.0	1013	0.5	0.6	0.4	+
19-9-27	7.0	1007	0.3	0.5	0.5	—
20-9-27	7.5	1010	0.4	0.6	1.1	+
21-9-27	7.5	1009	0.35	0.6	0.8	—
22-9-27	8.0	1009	0.4			+
23-9-27	7.0	1010			0.67	+

TABLE II

Date	Reaction pH	Sp Gr	Albumin per cent	Urea per cent	Fat per cent	Fibrin clot per cent
<i>Case No 2 (before breakfast urine)</i>						
19-10-27	5.0	1030	0.4	3.5	0.5	—
20-10-27	6.5		0.3		0.4	—
21-10-27	5.5	1027	0.24	3.2	0.5	—
22-10-27	5.5	1024	0.15	2.5	0.4	—
26-10-27	5.0	1024	0.12	2.9	0.2	—
27-10-27	5.0	1015	0.04	1.8	0.07	—
28-10-27	6.0	1013	0.05	1.4	0.1	—
29-10-27	6.0	1013	0.06	1.7	0.1	—
31-10-27	6.0	1013	0.01	1.3	0.03	—
1-11-27	5.5	1019	Nil	2.2	a trace	—
<i>Case No 2 (three hours after the midday meal)</i>						
19-10-27	5.0	1027	0.6	2.8	0.9	+
20-10-27	6.0	1010	0.06	1.4	0.09	—
21-10-27	8.0	1010	0.12	0.7	0.08	—
22-10-27						—
26-10-27	7.5	1013	0.07	0.9	0.1	—
27-10-27	8.0	1013	0.06	0.9	0.1	—
28-10-27	7.5	1008	0.07	0.7	0.07	—
29-10-27					0.03	+
31-10-27	8.0	1010	0.01	0.6		+

## DISCUSSION

Asa C Chandler (1924) gives a detailed description of the male and female of *Chandleriella bosci* (syn *Filaria bosci*) with various anatomical measurements

Four worms were obtained by him from the bird *Dissemurus paradiseus*, of which one was a female found lying on the peritoneal covering of the axillary air sac. A single male of *C bosci*, and two males of another species which he describes in the same paper as *F brevicauda*, formed the rest. Due to the presence of two kinds of microfilaria in the blood of the bird from which these worms were obtained and also because of the presence of another species of filaria in the same bird, Chandler expresses a doubt as already mentioned above, whether his description of the male and the female *Chandleriella* relates to the same species.

In the present case 13 worms were obtained, 4 males and 9 females, all from the heart. The description and the measurements of the male worm given by Chandler correspond closely with those of the worms found by us. As regards the female, his figures are widely at variance with ours. Table II gives the anatomical points in both the cases.

TABLE II

MALE			FEMALE		
Description	Chandler	Worm now described	Description	Chandler	Worm now described
Total length	9 to 11 mm	11.5 mm	Total length	28 mm	24.5 mm
Maximum diameter	150 $\mu$	145.4 $\mu$	Maximum diameter	730 $\mu$	327.0 $\mu$
Diameter head region	80 "	54.5 "	Diameter head region	160 "	63.6 "
Length of oesophagus	650 "	545.4 "	Diameter mid region		308.6 "
Nerve ring	135 "	134.0 "	Diameter anal region		118.6 "
Tip of tail to anus	140 to 160 "	160.0 "	Anterior end to nerve ring	200 $\mu$	138.0 "
Spicules length	70 to 80 "	84.0 "	Anus to post end	200 "	400.0 "
Maximum breadth	21 "	18.0 "	Length of oesophagus	980 "	700.0 "
			Genital opening from anterior end	450 "	310.0 "

This crow had a double infection, two kinds of microfilariae, a long form and a short form, being present in the blood. Adult worms found in the ventricle, however, all belonged to the same species. That the microfilaria (long form)

(2) *The determination of the chemical constants of the fat found in the urine studied in relation to the type of fat ingested*

A separate patient was placed under observation for this experiment Under this head were determined the constants of the excreted fat under

- (A) Ordinary hospital diet,
- (B) A diet containing cod-liver oil,
- (C) A diet containing vegetable ghee, and
- (D) A diet containing liquid paraffin administered in an emulsified form with gum acacia

We also noted the effects of the administration of calcium chloride on the amount of fat excreted (shown in Graph I, E), and the optical activity of the excreted fat after the administration of hydnocarpus oil This was carried out on a separate patient

The amount and type of fat ingested are shown in Graph I under sections A, B, C, D and E

The amount of fat in the hospital diet was calculated mostly from Sherman's tables, the exceptions such as Indian chicken, mutton, etc., were calculated from our own findings The chemical constants found under the varying conditions are tabulated in Tables IV, V, VI and VII

Fat constants on ordinary hospital dietary rich in cream

TABLE IV

	31-1-28	1-2-28		2-2-28		3-2-28
Iodine value	45.2	43.4	55.8	45.5	47	62.7
Butyrefractometer value at 40°C	50.3	48.5	48.7	48.6	47.3	50
Cholesterol, per cent	2.2	1.1	1.9	2	1	1.6
Reichert-Wollny value on a mixed sample		Figure obtained=1				
Melting point		30-40°C		38.5-39°C		

It may be noted that the figures obtained from this case show some variation from those obtained in Table III, possibly to be accounted for by the large amount of cream given in the dietary, the crystalline appearance of the fat being almost identical

The diet of the patient was now changed over to one containing 1 gramme of ordinary dietary fat and 6 grammes of cod-liver oil spread over six hours, followed by 23 grammes of ordinary dietary fat and another 6 grammes of cod-liver oil, and again 31 grammes of ordinary dietary fat and another 6 grammes of cod-liver oil for two successive days

Fat constants on a diet containing cod-liver oil,

PLATE LXXX



*Microfilaria* of *C. bosei* showing sheath

be noted that the figures again tend to resemble the figures obtained when on an ordinary hospital diet

After the 10th February, 1928, the vegetable ghee was stopped and an ordinary diet consisting of 32.5 grammes of dietary fat was given and in addition one ounce of liquid paraffin in gum acacia emulsion followed by 8.6 grammes of dietary fat and another ounce of liquid paraffin. The constants of the excreted fat on this diet were

TABLE VII

	13-2-28		14-2-28
Iodine value	67.8	74.6	62.6
Butyrefractometer value at 40°C	55	54.6	52
Cholesterol, per cent	2.7	3.1	3.1

No trace of the liquid paraffin could be obtained in the unsaponifiable matter of the excreted fat. This result agrees with the findings of Bloor and Connstein. This case was finally treated with 60 grains of calcium chloride, the result may be noted on the Graph I, E.

The administration of hydnocarpus oil

Another patient on ordinary hospital dietary was given on the first day of the experiment 50 minims of hydnocarpus oil. The optical activity of the sample was  $[\alpha]_D^{25}$  57.7 at 31°C, and the urine was collected for twenty-four hours. This was followed on the second day by the administration of 100 minims of the oil. Unfortunately, the administration of this oil could not be continued owing to the occurrence of acute symptoms of poisoning. The excreted fat was extracted from the forty-eight hours collection and examined for optical activity but with negative results. We admit that the amount of the oil was so small that we cannot draw the conclusion that the oil on absorption loses its optical activity.

### (3) *The effect of diet containing different fats on the excretion rate of the fat in the urine*

The object of these experiments was to endeavour to throw some light on the assimilation rate of the fat from the intestine as it appeared to us probable that the greater the assimilation rate from the gut the greater in proportion would be the excretion rate into the urine, other factors remaining constant. To eliminate variables such as fibrin clotting, etc., we carried out the experiment on three different patients.

*Case No. 1*—This patient was given first an ordinary hospital diet rich in cream for four days then a diet containing cod-liver oil for four days followed

# THE DISTRIBUTION OF THE BLOOD-GROUPS IN CERTAIN RACES AND CASTES OF INDIA

BY

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AND

M N LAHIRI, M B

[Received for publication, October 15, 1928]

HUMAN bloods are divided into four groups on the basis of two iso-agglutinins and two iso-agglutinogens. The formulæ of the four groups may be written as follows —

ab-o, b-A, a-B, o-AB corresponding to Jansky's groups 1, 2, 3 and 4, where capital A and B represent the two iso-agglutinogens, the small letters the two iso-agglutinins, and O, o the absence of iso-agglutinogens and iso-agglutinins respectively. The groups are usually designated as O, A, B and AB according to the agglutinin content of the erythrocytes.

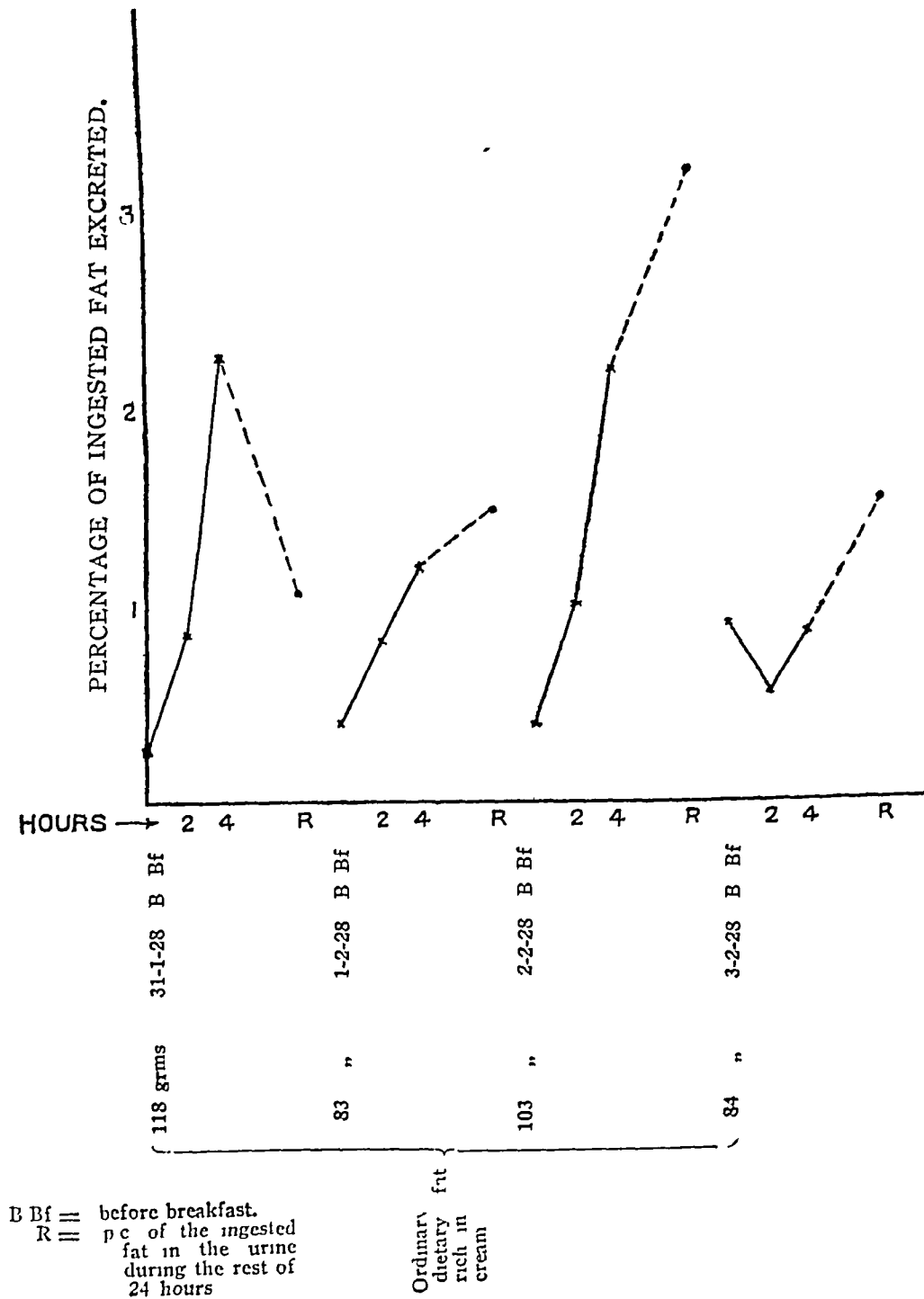
The significance of blood grouping in the study of the origin and relationships of different races of man was first pointed out by L. and H. Hirschfeld(1) as a result of the examination of soldiers of different nationalities stationed in Macedonia during the Great War.

The ratio of the agglutinin factor A in the corpuscles to B varies in different races and was called by them 'the biochemical race index'. On this basis these authors postulated the existence of three types of human races and showed that the ratio  $\frac{A + AB}{B + AB}$  is high among the European peoples, low among the peoples of Asia, especially in India, and that there is an intermediate type including such peoples as Arabs and Turks. The Hirschfelds suggested that A and B had different points of origin, namely, A in Europe and B in India and that there were two biochemical races which arose independently in different places.

The independent origin of B was deduced from the extremely low ratio of  $\frac{A + AB}{B + AB}$  found in the soldiers from India whom they examined and whom they indiscriminately labelled 'Indians'. It is impossible to say from the Hirschfelds' paper which of India's many races and castes were examined, but as it is known

by a diet containing vegetable ghee for two days and finally a diet containing liquid paraffin for two days The results of the experiment are shown in Graph II, under the sub-heads F, G, H and I

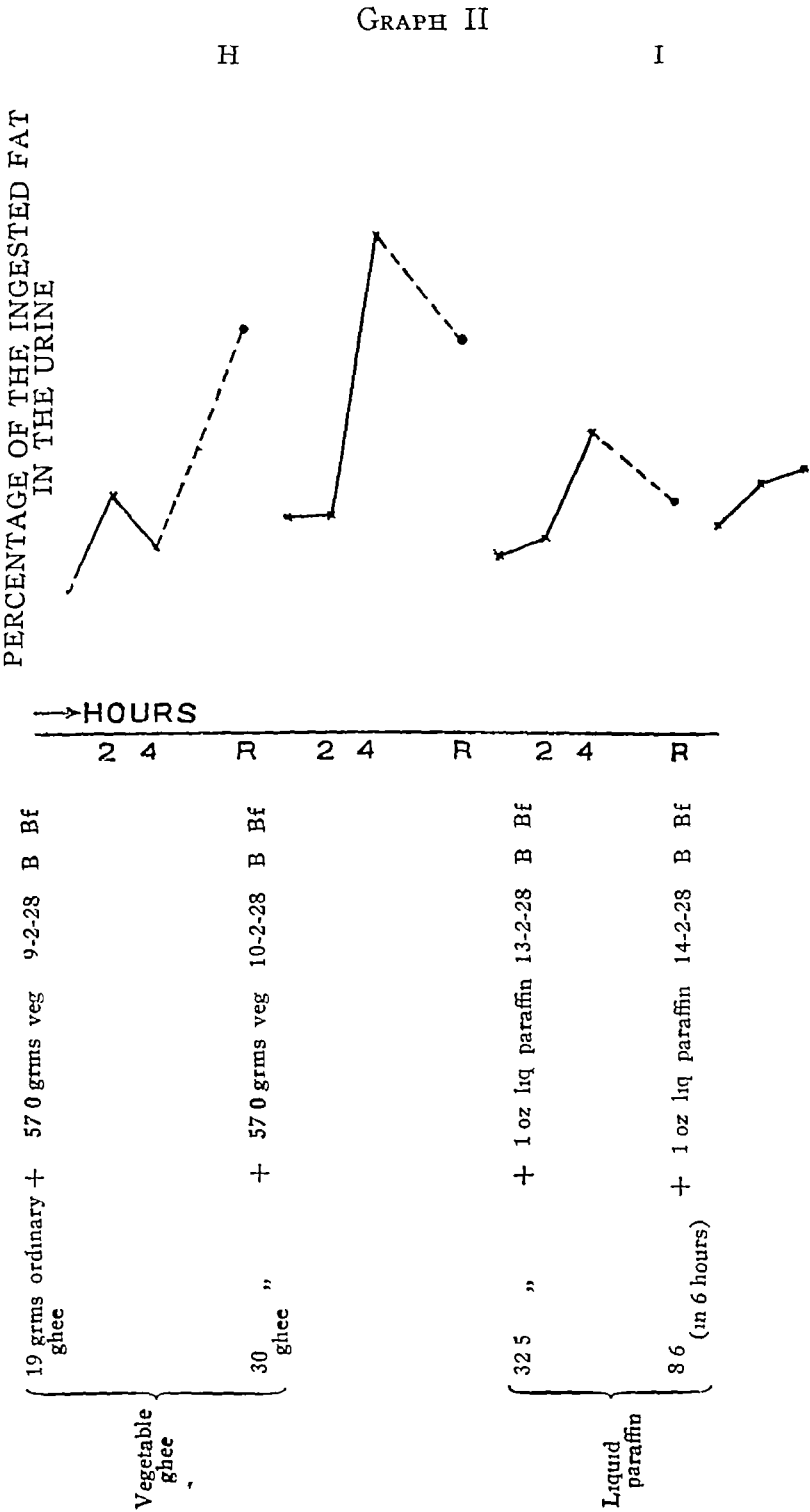
GRAPH II







ingested fat in the cream The percentage of fat in the urine before breakfast was calculated on the basis of the fat ingested on the previous day



B Bf == Before breakfast  
R == pc of the ingested fat in the  
urine during the rest of  
24 hours

consists of Uraons, Mundas and Santals, aboriginal peoples of Chota-Nagpur, who were either prisoners in the jails of that division or labourers recruited by the Tea Districts Labour Association at Ranchi (Chota-Nagpur Division)

The Turko-Iranian type (Plate LXXXI, figs 1 and 2) is represented by the Baluchis, Pathans and Hazaras of the Baluchistan Agency and North-West Frontier Province. They originated in the fusion of Turki and Persian elements: complexion fair, eyes dark, hair on face plentiful, head broad, nose moderately narrow, prominent and very long, stature above average.

The Indo-Aryan type (Plate LXXXI, figs 3 and 4) occupies the Punjab, Rajputana and Kashmir and to this type belong Jats, Rajputs and Khatri: stature mostly tall, complexion fair, eyes dark, nose prominent but not long.

The Dravidian type (Plate LXXXI, figs 5 and 6) extends from Ceylon to the valley of Ganges pervading the whole of Madras, Hyderabad, the Central Provinces and Chota-Nagpur. In this type the stature is below average, complexion very dark, approaching black, hair plentiful with occasional tendency to curl, head long, nose very broad, sometimes depressed at the root. The marked uniformity of their physical characters and their distinctive languages have led anthropologists to believe that they are the earliest inhabitants of India.

We shall speak of these 'types' as 'races'. The Turko-Iranian is a mixed race, the mixture having occurred in comparatively recent times. The caste system of India has preserved the purity of the Indo-Aryans and this is proved, so far as the Punjab is concerned, by the fact that 'to-day no trace of Dravidian blood is found among them, it seems that they are of foreign origin and all entered the Punjab with their own women' (7). The Dravidian is the most primitive race and is considered as being indigenous to India.

A large number of individuals not belonging to these three groups have also been examined. These were Hindus of various castes who came for treatment to the Pasteur Institute, Kasauli, from the United Provinces where, according to Risley, both high and low castes contain Dravidian blood admixed with Indo-Aryan.

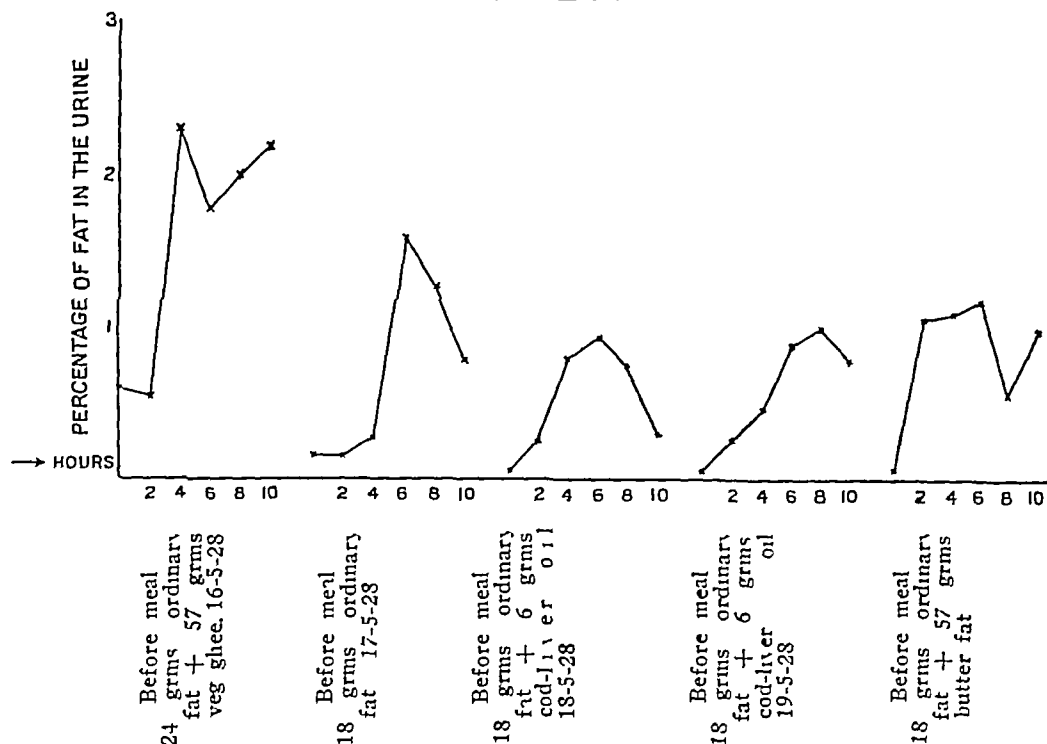
We have examined not more than one member of each family to exclude the possible preponderance of certain groups under the influence of family relationship and this is an important consideration in India where families are large and caste rules restrict marriages within certain caste limits.

### *Technique*

A measured amount of blood was drawn from the ball of the finger by puncture with a capillary pipette and expelled into 1.5 per cent citrated saline to make approximately a 5 per cent suspension of cells. A drop of the cell suspension was added to a drop of the two type sera containing the agglutinins *b* and *a* respectively on a porcelain slab with depressions. The slab was gently shaken for five minutes and a preliminary reading taken. It was left on the table for ten minutes, gently shaken and the final reading taken. The quantity used was large enough to prevent the readings from being obscured by the blood becoming dry.

19th, 18 grammes of ordinary fat were given and in addition 6 grammes of cod-liver oil. The results are shown in Graph IV. The estimations of the urinary fat were carried out as in the previous case.

GRAPH IV



The excretion rate under ordinary hospital diet and also when a special fat was added to the dietary appeared to be similar except on the first two days of the administration of cod-liver oil (Group IIJ) when the excretion rate was high.

Before concluding this paper, we wish to express our thanks to Dr. Sundar Rao, not only for supplying the necessary material but also for his co-operation and help in the work.

#### SUMMARY AND CONCLUSIONS

(1) We have shown very fully the chemical constants of the fat present in cases of chyluria and that when on an ordinary hospital diet these constants vary to some extent.

(2) We have also found that when cod-liver oil is administered there is a great deviation in these chemical constants particularly in the iodine value, butyrefractometer value and melting point of the fat. In these respects the constants approach those of cod-liver oil.

(3) We have failed to obtain any trace of liquid paraffin in the excreted fat when the diet contained this ingredient in the form of an emulsion with gum acacia.

and the Turko-Iranian the lowest of B, the frequency of B decreases as we travel up the Ganges valley to the North-West Frontier, the Hindus of the United Provinces standing in an intermediate position between the Dravidians and the Indo-Aryans in this regard. These findings are quite in accordance with what might be expected from the views expressed by Risley with regard to the origin and relationship of these types. The same order holds good for O and AB, the Dravidians having the lowest percentage of O and the highest of AB, while the Turko-Iranian has the highest of O and the lowest of AB.

The frequency of A is also highest in the Dravidian and lowest (except for Hindus of the United Provinces) in the Turko-Iranian. With regard to the low frequency of A amongst Hindus from the United Provinces, a figure between those obtaining for Indo-Aryans and Dravidians might have been expected since these Hindus are an admixture of the two above races, but this is not the case no explanation of the discrepancy can be offered at present.

The low frequency of A and the high frequency of B in these three races of India as compared with the control group of British soldiers, confirm the findings of other workers with regard to Asiatic peoples in general. Our figures, however, do not support the theory that B originated in India and A in Europe since although the primitive indigenous race (Dravidian) shows the highest frequency of B, it also shows the highest frequency of A in our series in spite of the fact that this race has always been strictly isolated from and never inter-married with the foreigners who invaded India in successive waves from the earliest Aryan times. We must conclude that the comparatively high frequency of A in the Dravidian cannot be due to admixture with other races reaching India in the days of the Aryans or later, but was already a characteristic of this race when the first Aryan invaders appeared on the scene. In view of the work of Landsteiner and Miller(8) on the differentiation of human and anthropoid bloods, it might be conceived that both agglutinogens A and B arose in India or in some other portion of the great Indo-African continent not in man, but in the common ancestors of the anthropoids and man.

### CONCLUSIONS

1 The 'biochemical race index' is an inadequate measure of racial differences based on blood grouping.

2 The calculation of the true frequencies of the agglutinogens A, B and O shows that amongst certain well defined races in India there is a distinct relation between anthropological characters and the frequency distribution of the four blood groups.

3 The three races examined fall within the Asiatic group of other writers, the Dravidian possessing the highest frequencies of both A and B.

4 The frequencies of both A and B tend to diminish and, naturally, that of O to increase in the races encountered as one travels up the Ganges valley to the North-West Frontier of India.



# OBSERVATIONS ON THE MEDICO-LEGAL APPLICATION OF BLOOD GROUPING WITH A NOTE ON BLOOD-GROUPS IN A POLYANDROUS FAMILY

BY

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THE four blood groups have, for some years, been the subject of study from a medico-legal point of view. The medico-legal application of blood grouping is based on the fact that an agglutinin never appears in the blood of an individual unless it is present in one of the parents. It has been widely applied in cases of disputed parentage in certain countries.

Ottenburg(1 and 2) considers that blood groups are inherited in accordance with Mendelian laws on the basis of two independent pairs of factors, the iso-agglutinogens A and B being dominant to their respective iso-agglutinins a and b, and, according to him, the genetic formulæ of the four blood groups are —

Group	O	=	aabb
„	A	=	AAbb Aabb
„	B	=	aaBB aaBb
„	AB	=	AABB AaBB AABb AaBb

In 1925 Bernstein formulated the hypothesis that blood-groups are inherited as a series of three multiple allelomorphs. He assumes that the iso-agglutinogens A and B are dominant to the same recessive (R) (this hypothesis has been confirmed by Snyder)(3). Since the dominant factor may carry the recessive R, the genetic formulæ of the four blood groups are —

Group	O	=	RR
„	A	=	AA or AR
„	B	=	BB or BR
„	AB	=	AB

A NEW VARIETY OF PROTANOPHELINE *A BARBIROSTRIS*  
VAN DER WULP, VAR *AHOMI*, FOUND IN UPPER ASSAM

BY

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[Received for publication, November 13, 1928]

WHILE malaria-surveying (1927) over some tea gardens in Upper Assam, I found a new type of anopheline larva which resembled *A barbirostris* Van der Wulp, and the mosquitoes bred from these larvæ were similar to *A barbirostris*. The larvæ then struck me to be but a local variation of this species, but this year again I have obtained many specimens from different places in the Lakhimpur district of Assam, and I now consider them to belong to a new variety of *A barbirostris* and accordingly name it *A barbirostris* var *ahomi*.

I have subsequently examined the characters of the male genitalia of a few of the adults bred out of these larvæ and found them to be almost similar to those of typical *barbirostris*, except in the characters of the thecal leaflets which are rather longer and more regular in arrangement on the thecal knob than in the type species.

The following is a description of the larva —

HEAD

*Antenna* carries a branched hair on its inner side slightly below its middle.

*Clypeal hairs*—Anterior internal—long, straight, and has several branches on its distal half (*see* figure below). Both the hairs are placed close together as in other protanophelines.

Anterior external—dendriform branching with more than 50 to 60 twigs.

Posterior—short, straight and usually bifid, but simple and trifid hairs have also been seen. These branches do not come out of the root as in the typical *barbirostris*, but are formed by splitting the terminal half into two or three divisions.

*Frontal hairs*—They are long, profusely branched and much pigmented.

The only exceptions to the hypothesis of three multiple allelomorphs were as follows —

Family No	Crosses	Offspring
20	A $\times$ AB	O, A
31	O $\times$ A	O, AB
48	A $\times$ A	A, AB

Regarding families 31 and 48 the AB offspring cannot be a legitimate issue according to either of the hypotheses, but no history was obtained as these two families were among the recruits of the Tea District Labour Association and could not be further traced. The offspring O in family No 20 (father A, mother AB) satisfies Ottenburg's hypothesis, but not that of Bernstein. The history of the family is, however, as follows —

This family came to the Pasteur Institute, Kasauli, for anti-rabic treatment from a village in the Simla district. Samples of blood from all the members of the family were tested and as the result of the test in the case of one child was considered to be anomalous the head of the family was questioned. At first he denied the possibility of his not being the father of the child but later on made a frank confession stating that his present wife was really his younger brother's wife although both of them had access to her as is their custom. The child (A) was undoubtedly his as it was born more than a year after his brother's death but there was every doubt regarding the other child (O).

Instances of this type of polyandry are not uncommon in India, according to Risley(5), who cites two well recognized types of polyandry in the Census Report for India, 1901, the matriarchal, when a woman forms alliances with two or more men who are not related to one another, and the succession is therefore traced through the female, and the fraternal when she becomes the wife of several brothers. The former at the present day is confined to the Todas of the Nilgiris and the Nayars and other castes of the Malabar coast and is now gradually falling into disrepute, but where it exists, it is gradually taking the fraternal form. The latter is still more or less common along the whole of the Himalayan area from Kashmir to the eastern extremity of Assam, but is also apparently falling into disrepute. The family just described is an example of fraternal polyandry in hill peoples.

#### SUMMARY

The results of these tests do not afford evidence in support of either of the two rival hypotheses regarding the inheritance of blood groups, but the observations are recorded to show an apparent exception to Bernstein's hypothesis which on



hairs bearing a small number of branches (11 to 22 against 60 or more in the type) like *umbrosus*, and in the posterior clypeal hairs being bifid and relatively long, but its internal clypeals were not frayed as in *ahom*. The following table shows the differential characters of these three types of larvæ —

Clypeal hairs	<i>A barbirostris</i> Van der Wulp	<i>A barbirostris</i> var <i>pallidus</i> Swellengrebel and Swellengrebel vel <i>A barbumbrosus</i> Strickland	<i>A barbirostris</i> var <i>ahom</i>
Anterior internal	long straight and simple	long straight and simple	long, straight and pin-nately branched on its distal half
Anterior external	dendriform branching with 60 or more branches	dendriform branching with 11 to 22 branches	dendriform branching with more than 50 to 60 branches
Posterior	a small tuft of three or more branches	bifid and relatively long	simple, bifid or trifid and short

The justification for naming it a variety is the fact that annectant forms with the type species do not occur, and that in some '*barbirostris* regions,' e.g. Malay, the characteristics of the variety are not seen.

I beg to thank Dr C Strickland, M A, M D, Professor of Medical Entomology, for his kind help in this matter.

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# CHYLURIA SOME BIOCHEMICAL ASPECTS.

BY

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AND

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[Received for publication, November 8, 1928]

We were fortunate in obtaining the urine from a number of patients suffering from this condition which afforded us the opportunity of making several chemical observations on the fat contents. These we consider are of sufficient interest for publication. All the patients were admitted to the Carmichael Hospital for Tropical Diseases, Calcutta, and were under the immediate care of Dr Sundar Rao.

General nature of the observations —

(1) The physical condition, amount and chemical nature of the fat present in cases of chyluria

(2) The determination of the chemical constants of the fat present in the urine studied in relation to the type of fat ingested

(3) The effect of diet containing different fats on the excretion rate of the fat in the urine

(1) *The physical condition, amount and chemical nature of the fat present*

Two patients admitted into the hospital were taken for this investigation. Two specimens of urine were examined each day from each case, one specimen before breakfast and another three hours after the midday meal. The results of these examinations are given in Tables I and II.

effect of activated sludge on pathogenic organisms or on any selective action on the various members of the coli group

The experiments detailed below were made for the purpose of investigating the following points —

(1) Whether the reduction in the *B coli* content is consistent, and at what rate it is brought about

(2) Whether there is any selective action on the members of the *B coli* group

(3) The germicidal action on pathogenic organisms of the intestinal group

(1) *The reduction of B coli and the rate at which this is accomplished*

Raw Calcutta sewage was used throughout

Decimal dilutions were made to determine the coli content

The sewage was mixed with 15 per cent of activated sludge and aerated, for periods varying from 1 to 24 hours

After aeration, the sewage was allowed to settle for 10 minutes, when the coli content was again determined

The results are given in the table following —

After aeration of	<i>B coli</i> present in	Percentage reduction
1 hour	0 000001 c c	60 (determined by sub-dilution)
2 hours	0 000001 „ raw sewage 0 00001 „ effluent	90
3 hours	0 0000001 „ raw sewage 0 00001 „ effluent	90
4 hours	0 0000001 „ raw sewage 0 00001 „ effluent	99
5 hours	0 0000001 „ raw sewage 0 001 „ effluent	99
6 hours	0 0000001 „ raw sewage 0 001 „ effluent	99.9
24 hours	0 0000001 „ raw sewage 0 01 „ effluent	nearly 100

It will be seen that there is a progressive bactericidal action proportional to the time of aeration. With 4 hours' aeration there is a very considerable bacterial purification approximating to the chemical purification which takes place in this time. With 24 hours' aeration the bactericidal action is pronounced.

In both patients there is a great variation in the fat and albumin content varying from a trace to 1.1 per cent in the case of the fat and from nil to 0.6 per cent in the case of albumin. We expected to find a closer relationship between the fat and albumin figures and also higher figures for the fat in the specimens taken three hours after meals. As regards the protein content of the urine, both globulin and albumin were found to be present. It was also noted that fibrin was more frequently present in the specimens collected after the midday meal.

Physical condition of the fat —

This existed in the urine in very minute globules, the size of the globules was estimated for us by Dr. Maplestone and was found to vary, the largest were in the neighbourhood of  $6\ \mu$ , but the majority measured  $0.25\ \mu$  or less.

Chemical nature of the fat —

Sufficient fat was collected from each patient separately to allow a partial determination of the chemical constants. The results of this examination are shown in Table III.

TABLE III

	Case 1	Case 2
Melting point	36—37°C	36—37°C
Iodine value	63.8	61.6
Saponification value	195	202
Acid value	10	8
Butyrefractometer value at 40°C	51.5	53.7
Cholesterol, per cent	2.4	3.8
Ash, per cent	0.1	.

Both patients were on ordinary hospital diet consisting of milk, bread, sugar, rice, dal, eggs, potatoes, chicken, green vegetables, fish and ghee. The fat was extracted from the urine by means of ether, several extractions being carried out. From the low figure obtained for the ash coupled with the fact that on acidification of the residue after complete ether extraction of the sample and re-extraction with ether no fatty acid was obtained, it must be concluded that no fat in the form of soap was present. The iodine value was determined by the Hanus method and the cholesterol content by using the Liebermann-Burchard colour reaction. The figures obtained from the two cases show a very marked similarity, the fat excreted in both cases also exhibited the same crystalline appearance, a microphotograph of one specimen only is reproduced (Plate LXXXII).

(3) The actual germicidal effect on cultures of pathogenic organisms was further investigated, *V. cholerae*, *B. typhosus* and *B. paratyphosus* being used. Emulsions of 24 hours' agar cultures were taken and their number per c.c. examined by Brown's opacity method. A definite quantity of emulsion was then put in weak sewage, so that the fluid now contained 10 millions per c.c. This was corroborated by examination by the dilution method.

The sewage containing the organisms was aerated along with 15 per cent of activated sludge. At the end of each hour, ten minutes' settlement was given and 1 c.c. of the supernatant fluid examined for the specific organisms. The results are given in the table below —

#### EXPERIMENTS WITH *B. typhosus*

In raw sewage	<i>B. typhosus</i> present in 0.0000001 c.c.					
After 1 hour's aeration	"	"	"	"	0.0000001	60 per cent reduction (determined by sub-dilution)
" 2 hours'	"	"	"	"	0.000001	} 90 per cent reduction
" 3 "	"	"	"	"	0.000001	
" 4 "	"	"	"	"	0.000001	
" 5 "	"	"	"	"	0.000001	} 99 " "
" 6 "	"	"	"	"	0.00001	
" 24 "	"	"	"	"	0.001	nearly 100 " "

*V. cholerae* and *B. paratyphosus* A gave similar results. From these we conclude that aeration with activated sludge has a very definite bactericidal action on pathogenic intestinal organisms and that 6 hours' aeration was sufficient to give a very large reduction in these organisms.

#### SUMMARY

1 The activated sludge process exerts a definite and consistent bactericidal action on the coli group of organisms. The rate has been determined.

2 The action is selective, Class I and Class II (Clemesha) are reduced and the proportion of Class III is accordingly increased.

3 The process exerts bactericidal action on *B. typhosus*, *B. paratyphosus* A and *V. cholerae*.

4 In these experiments, 6 hours' aeration with 15 per cent of activated sludge is sufficient to produce for preventive purposes the maximum action.

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TABLE V

	4-2-28	6-2-28			7-2-28			8-2-28
Iodine value	56.1	50.8	64	61.4	80	93	147.8	88
Butyrefractometer value at 40°C	56.2	50.6	55.6	55.9	60	60	60.3	58.2
Cholesterol, per cent	3.9	4.7	3.3	2	3.1	2.8	3.2	2.3
Reichert-Wollny value on a mixed sample		Figure obtained = 0.8						
Melting point		Sample liquid						

The iodine value of the cod-liver oil administered to the patient was 156.4 and the butyrefractometer value at 40°C, 73.6. It is of interest to note the change in the iodine values, the increase in the butyrefractometer readings and cholesterol contents, and the great variation in the melting point of the excreted fat. A peculiar odour similar to cod-liver oil was also observed. From these findings it would appear that feeding with a substance like cod-liver oil caused a marked change in the constants of the fat after absorption through the gut.

On the 9th February, 1928, the administration of cod-liver oil was stopped and in addition to 19 grammes of ordinary dietary fat 57 grammes of vegetable ghee were given followed by 30 grammes of dietary fat and another 57 grammes of vegetable ghee. The constants of the vegetable ghee administered were, iodine value 65.1, butyrefractometer value at 40°C, 49.5. Melting point 39°C. The analytical results obtained from the urinary fat on this diet were

Fat constants on a diet rich in vegetable ghee

TABLE VI

	9-2-28			10-2-28	
Iodine value	68.4	67.5	68.2	66	67
Butyrefractometer value at 40°C	53.1	54.3	52.5	52	52
Cholesterol, per cent	3.6	3.9		1.8	1.7
Melting point.	32-35°C				

Our object in switching over to a diet containing a large proportion of vegetable ghee was to note the effect on the excreted fat after the administration of the cod-liver oil when the patient was again put on a diet in which the constants of the major portion of the fat were known. From the results obtained it may

	0 hour	1 hour	2 hours	3 hours	4 hours	25 hours	49 hours
Filtered and unboiled water	2,500	1,500	1,000	500	0	0	0
Filtered and boiled water	5,000	4,000	6,000	10,000	6,000	10,000	36,000

Hankin thought that the vibriocidal action of the Jumna river water was due to the presence in it of a volatile substance D'Herelle (1916) (2) doubts this explanation and suggests the vibriocidal action to be due to the presence of bacteriophage

Hankin also stated that he was never able to demonstrate that the ingestion of water of these rivers was responsible for the development of a single case of cholera We cannot agree with this statement because in Hardwar at least we were able to trace most of the cases of cholera to Ganges water, Khan (1928) (3)

At Allahabad the Jumna joins the Ganges and the Union, called the *Sangam*, is a place of special sanctity Every year in the month of January a fair of considerable size takes place, which is called the *Magh Mela* At the time of the *Magh Mela* of 1928 we established a laboratory on the *Mela* site at the *Sangam* We carried out experiments on the spot on the duration of life of vibrios in the water of the rivers Ganges and Jumna (*see* Protocol below)

Water from the Ganges, the Jumna and a well in the *Mela* site was used, also water from the *Sangam* and an artificial *Sangam* made by mixing equal quantities of Ganges and Jumna water Water from each source was divided into four parts, one part used untreated (as it came from its source), the second was filtered only, the third was boiled only (5 minutes) and the fourth was boiled and filtered The filtration was done through filter paper One hundred cubic centimetres of each sample were placed in Ehrlenmeyer flasks of 200 c c capacity To one set of these flasks was added an agglutinable vibrio isolated from a case of cholera at Hardwar in 1927, and to another similar set was added an inagglutinable vibrio isolated from the river Ganges at Hardwar in the same year A 24 hours' agar slope culture of either vibrio was suspended in 10 c c of normal saline and 0.111 mg of the vibrios were added to each flask

Distilled water to which vibrios were added and water from each source to which no vibrios were added were used as controls

The flasks were kept at room temperature From the next day, a sample measuring 1 c c was taken from each flask daily and inoculated into 9 c c of 1 per cent peptone water pH about 8 After 24 hours' incubation at 37°C an ordinary size loopful was plated out on bile salt agar plates pH 8 and incubated in the same way Characteristic colonies were fished out and examined for vibrios If vibrios were not found, the sample was considered as negative The results are given in the table below





## EXPERIMENT I

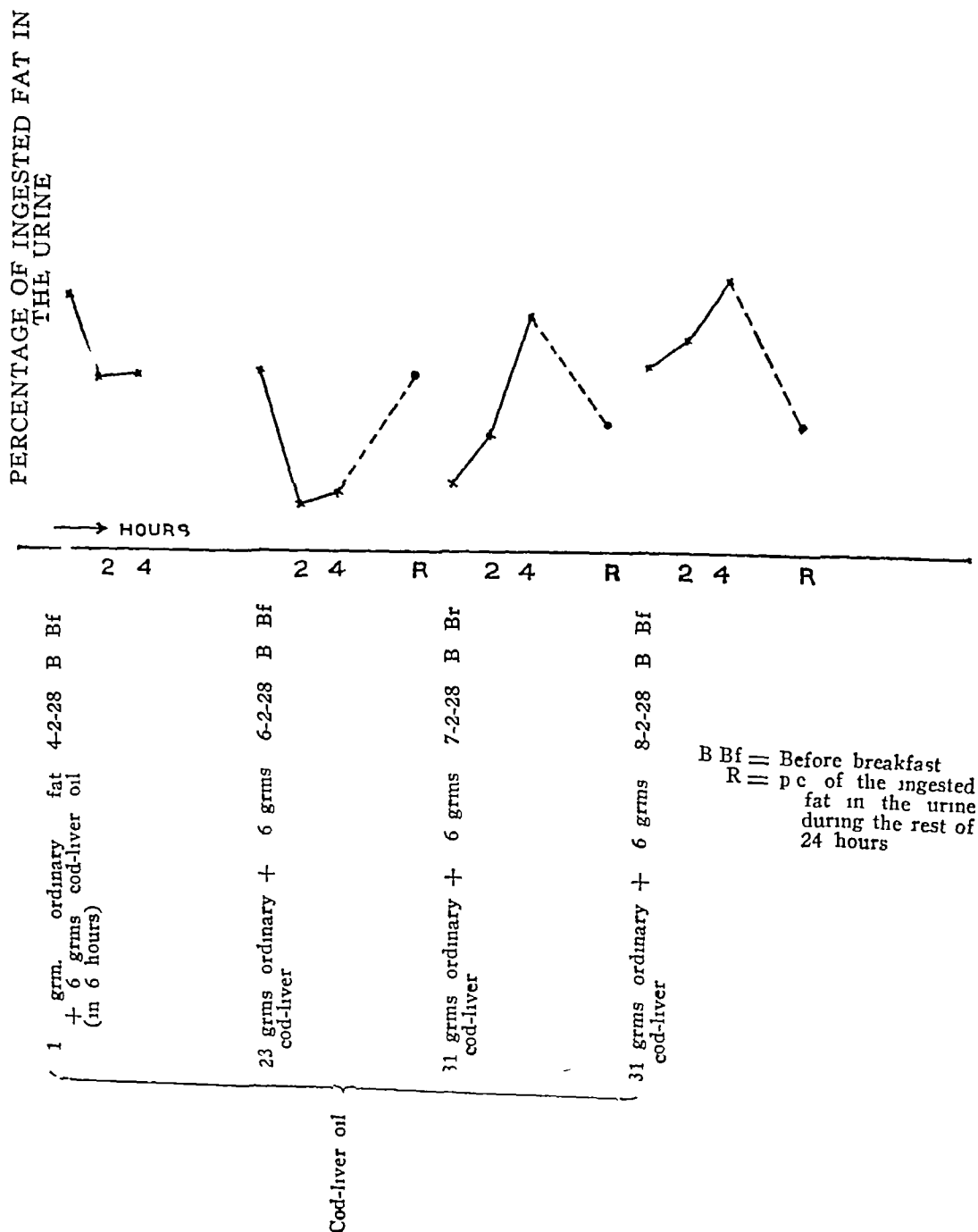
	Source of water	PERCENTAGE OF POSITIVE SAMPLES		LAST DAY OF POSITIVE SAMPLES, I.E., DURATION OF LIFE	
		Agglutinable vibrio	Inagglutinable vibrio	Agglutinable vibrio	Inagglutinable vibrio
Ganges water	Raw water	25.0	50.0	8	8
	Raw but filtered	25.0	100.0	8	8
	Boiled	0	80.0	0	10
	Boiled and filtered	25.0	83.3	8	12
Jumna water	Raw water	0	75.0	0	8
	Raw but filtered	0	75.0	0	8
	Boiled	33.3	100.0	6	12
	Boiled and filtered	11.1	71.4	18	14
Saugam	Raw water	22.2	75.0	18	8
	Raw but filtered	14.3	60.0	14	10
	Boiled	71.4	77.7	14	18
	Boiled and filtered	22.2	75.0	18	16
Mixture, i.e., equal quantities of Ganges and Jumna water	Raw water	33.3	100.0	6	8
	Raw but filtered	50.0	100.0	12	4
	Boiled	60.0	83.3	10	12
	Boiled and filtered	23.0	66.6	27	12
Well water	Raw water	0	66.6	0	12
	Raw but filtered	33.3	100.0	6	6
	Boiled	11.1	88.8	18.	18
	Boiled and filtered	0	53.8	0	26
	Distilled water	25.0	0	8	0

vibrios We, therefore, fixed the limit of ten days, i.e., if vibrios were not isolated from any one of the 42 samples, taken daily for 10 successive days, this was taken as sufficient reason to presume

The procedure adopted in these cases was to determine the urinary fat before breakfast, then to note the amount of fat given with the meal and also the amount of any special fat administered and to estimate the percentage of the

GRAPH II

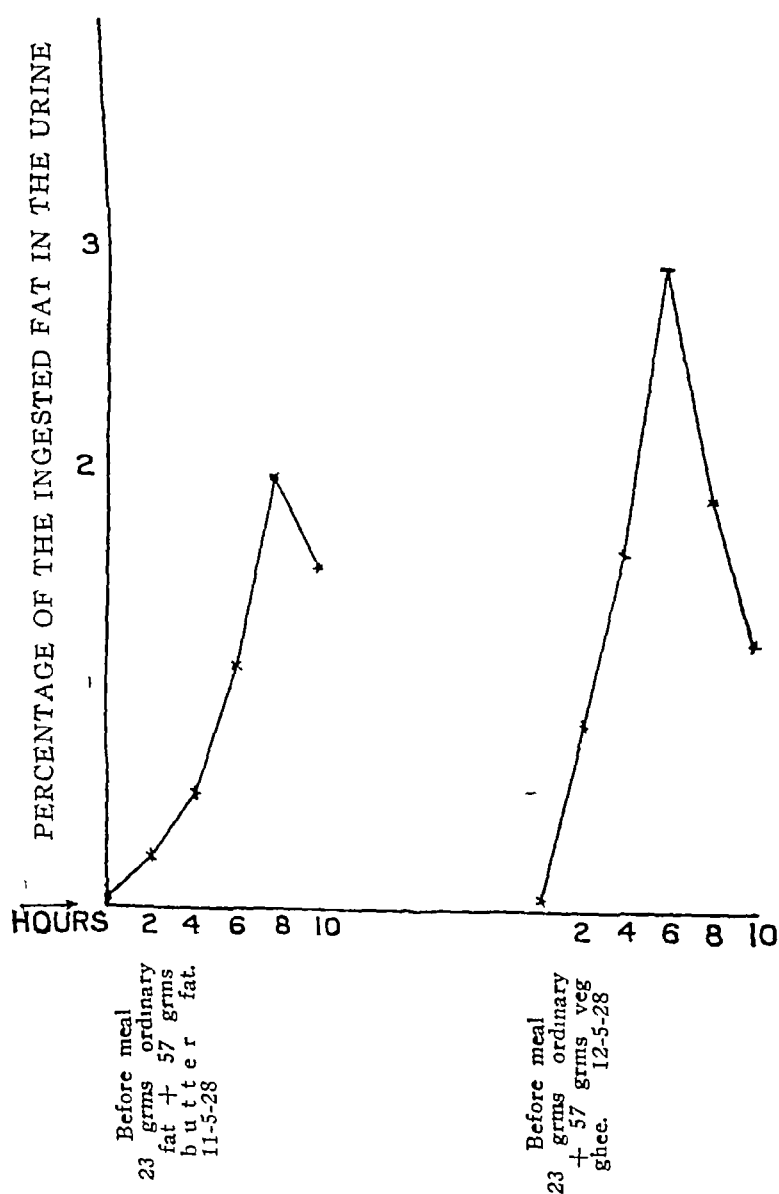
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*Case No 2*—In addition to 23 grammes of ordinary dietary fat, the patient was given 57 grammes of butter fat on the first day followed by 57 grammes of vegetable ghee on the next day. The urinary fat was estimated before food and again, 2, 4, 6, 8 and 10 hours after administration. The results of this experiment are shown in Graph III.

GRAPH III



*Case No 3*—This patient was given 24 grammes of ordinary dietary fat and 57 grammes of vegetable ghee on the 16th May, 1928, followed by an ordinary diet containing 18 grammes of fat on the 17th May, 1928. On the 18th and

Duration of life of the agglutinable vibrio in water given below      Duration of life in waters of which the percentage of positive samples is as low as 25 per cent or less has been taken as zero

TABLE III

Source of water	EXPERIMENT I				EXPERIMENT II				EXPERIMENT III				EXPERIMENT IV			
	Last day of positive samples, i.e., duration of life	Number of positive samples in the total	Percentage of positive samples		Last day of positive samples, i.e., duration of life	Number of positive samples in the total	Percentage of positive samples		Last day of positive samples, i.e., duration of life	Number of positive samples in the total	Percentage of positive samples		Last day of positive samples, i.e., duration of life	Number of positive samples in the total	Percentage of positive samples	
Ganges	0	0	0		8	2 in 4	50.0		0	0	0		0	0	0	
Raw water	0	0	0		18	3 in 9	33.3*		0	0	0		0	0	0	
Raw but filtered	0	0	0		2	1 in 1	100.0		2	1 in 1	100.0		5	3 in 3	100.0	
Boiled	0	0	0		0	0	0		2	1 in 1	100.0		5	3 in 3	100.0	
Boiled and filtered	0	0	0		0	0	0		2	1 in 1	100.0		5	3 in 3	100.0	
Jumna	0	0	0		0	0	0		0	0	0		0	0	0	
Raw water	0	0	0		8	2 in 4	50.0		0	0	0		0	0	0	
Raw but filtered	6	1 in 3	33.3		29	7 in 15	46.6		0	0	0		5	3 in 3	100.0	
Boiled	0	0	0		10	2 in 5	40.0		2	1 in 1	100.0		4	2 in 2	100.0	
Boiled and filtered	0	0	0		0	0	0		0	0	0		0	0	0	
Raw water	0	0	0		0	0	0		0	0	0		0	0	0	
Raw but filtered	14	5 in 7	71.4		2	1 in 1	100.0		7	2 in 2	100.0		5	3 in 3	100.0	
Boiled	0	0	0		14	3 in 7	42.8		8	2 in 3	66.6		5	3 in 3	100.0	
Boiled and filtered	0	0	0		0	0	0		0	0	0		0	0	0	
Raw water	6	1 in 3	33.3		0	0	0		0	0	0		0	0	0	
Raw but filtered	12	3 in 6	50.0		0	0	0		2	1 in 1	100.0		0	0	0	
Boiled	10	3 in 5	60.0		0	0	0		2	1 in 1	100.0		7	4 in 5	80.0	
Boiled and filtered	0	0	0		0	0	0		2	1 in 1	100.0		5	3 in 3	100.0	
Mixture	0	0	0		0	0	0		0	0	0		0	0	0	
Raw water	0	0	0		0	0	0		0	0	0		0	0	0	
Raw but filtered	6	1 in 3	33.3		0	0	0		2	1 in 1	100.0		0	0	0	
Boiled	0	0	0		0	0	0		9	2 in 4	50.0		5	3 in 3	100.0	
Boiled and filtered	0	0	0		0	0	0		8	3 in 3	100.0		5	3 in 3	100.0	
Distilled water	0	0	0		0	0	0		0	0	0		0	0	0	

\* For calculating the mean, etc. this although a little above 25 is also taken as zero owing to the extraordinary long duration of life

PLATE LXXXII

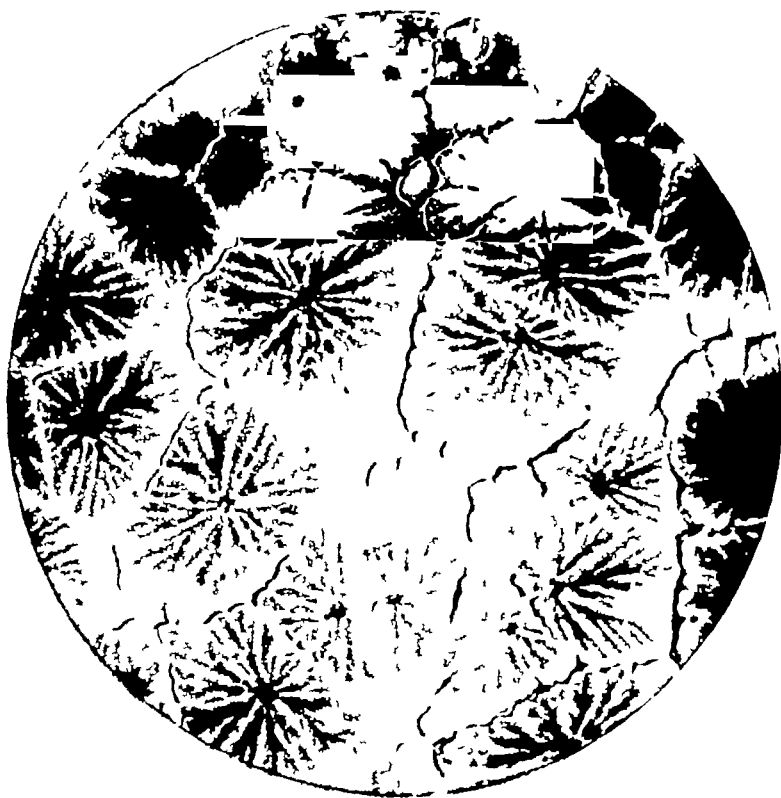


TABLE V

Duration of life in each water and in each experiment

Source of water			EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		EXPERIMENT IV		Mean of all four experiments (filtered)	Mean of all four experiments (unfiltered)
			Filtered water	Unfiltered water	Filtered water	Unfiltered water	Filtered water	Unfiltered water	Filtered water	Unfiltered water		
INAGGLUTINABLE VIBRIO	Ganges	Boiled	12	10	10	14	10	9	2	6	8.75	7.625
		Unboiled	8	8	20	14	0	0	8	0		
	Jumna	Boiled	14	12	14	18	11	10	4	4	7.375	6.75
		Unboiled	8	8	8	0	0	2	0	0		
	Sangam	Boiled	16	18	20	18	7	10	0	4	9.0	9.125
		Unboiled	10	8	14	10	2	2	3	3		
	Mixture.	Boiled	12	12	2	0	8	10	4	4	3.75	4.625
		Unboiled	4	8	0	0	0	0	0	3		
	Well	Boiled	26	18	0	0	9	10	4	3	6.25	6.125
		Unboiled	6	12	0	0	2	2	3	4		
AGGLUTINABLE VIBRIO	Ganges	Boiled	0	0	0	2	2	2	5	5	0.875	2.125
		Unboiled	0	0	0	8	0	0	0	0		
	Jumna	Boiled	0	6	10	29	2	0	4	5	3.0	5.0
		Unboiled	0	0	8	0	0	0	0	0		
	Sangam	Boiled	0	14	14	2	8	7	5	5	3.375	3.5
		Unboiled	0	0	0	0	0	0	0	0		
	Mixture.	Boiled	0	10	0	0	2	2	5	7	2.625	3.125
		Unboiled	12	6	0	0	2	0	0	0		
	Well	Boiled	0	0	0	0	8	9	5	5	2.625	1.75
		Unboiled	6	0	0	0	2	0	0	0		

(4) We have also failed to find any optically active fat after the administration of hydnocarpus oil

The expenses incurred over this work have been defrayed from a grant received from the Indian Research Fund Association



TABLE VI

*Duration of life in boiled and unboiled water of all experiments*

Source of water		EXPRIMENT I		EXPERIMENT II		EXPERIMENT III		EXPERIMENT IV		Mean of all experiments	
		Boiled.	Unboiled	Boiled	Unboiled	Boiled	Unboiled	Boiled	Unboiled	Boiled	Unboiled
INAGGLUTINABLE VIBRIO	Ganges	12 10	8 8	10 14	20 14	10 9	0 0	2 6	8 0	9 125	7 25
	Jumna	14 12	8 8	14 18	8 0	11 10	0 2	4 4	0 0	10 875	3 25
	Sangam	16 18	10 8	20 18	14 10	7 10	2 2	0 4	3 3	11 625	6 5
	Mixture	12 12	4 8	2 0	0 0	8 10	0 0	4 4	0 3	6 5	1 875
	Well	26 18	6 12	0 0	0 0	9 10	2 2	4 3	3 4	8 75	3 625
	Mean	15	8	9 6	6 6	9 4	1 0	3 5	2 4		

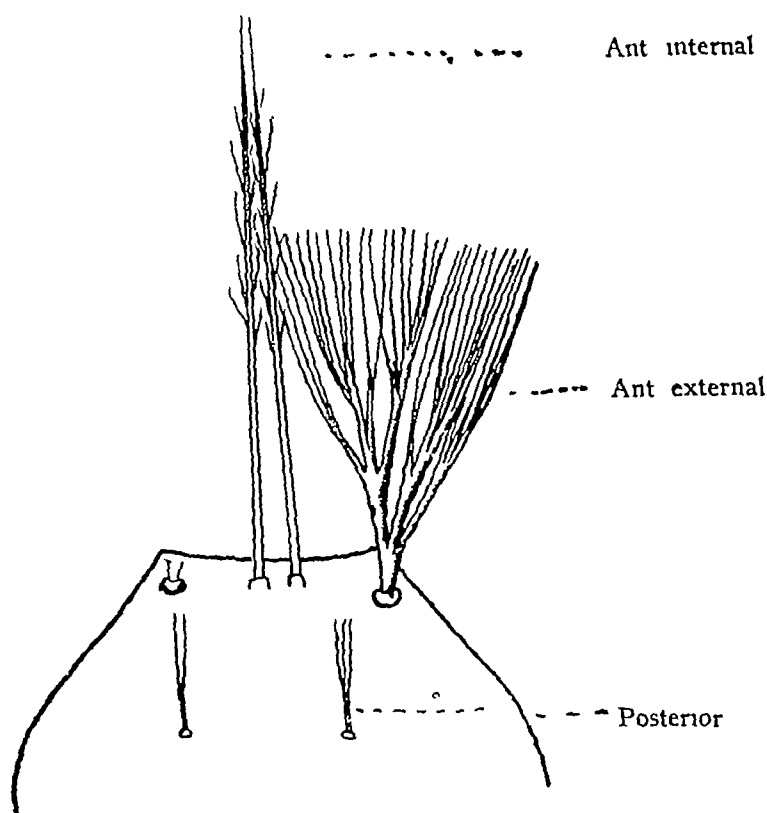
AGGLUTINABLE VIBRIO	Ganges	0 0	0 0	0 2	0 8	2 2	0 0	5 5	0 0	2 0	1 0
	Jumna	0 6	0 0	10 29	8 0	2 0	0 0	4 5	0 0	7 0	1 0
	Sangam	0 14	0 0	14 2	0 0	8 7	0 0	5 5	0 0	6 875	0
	Mixture	0 10	12 6	0 0	0 0	2 2	2 0	5 7	0 0	3 250	2 5
	Well	0 0	6 0	0 0	0 0	8 9	2 0	5 5	0 0	3 375	1 0
	Mean	3 0	2 4	5 7	1 6	4 2	4	5 1	0		

*Occipital hairs*—Both the internal and the external are branched, but the internal is thicker and larger than the external

*Mentum*—Shows seven teeth, the third from its base on either side being very small

*Thorax*—This bears a pair of well-developed palmate hairs

The thoracic pilosity (pro-, meso-, and meta-thoracic group of hairs) as recently described by Puri (1928) has been studied by me in all the larvæ of this variety, and found to be similar to those found on typical *A. barbirostris* Van der Wulp



clypeal hairs of  
*A. barbirostris* var *ahomii*

*Abdomen*—This carries seven pairs of well-developed protanopheline type of palmate hairs, the first two pairs being slightly smaller in size

*Dorsal plaques* are oval in shape and elongated laterally, and gradually increase in size from the first to the seventh segment

#### REMARKS

Specimens from the Malay Archipelago have been described and named a variety of *barbirostris* by Swellengrebel under the name of *M. barbirostris* var *pallidus*, but have been renamed by Strickland as a distinct species, *A. barbumbrosus*. This species differed from *barbirostris* in the external clypeal

the latter for  $45 \pm 58$  days. This difference of  $487 \pm 87$  days is certainly significant being more than 5 times its probable error. In unboiled water the inagglutinable vibrio lived for  $45 \pm 52$  days and the agglutinable vibrio lived for  $11 \pm 29$  days. The difference of  $34 \pm 6$  days is certainly significant as it is more than 5 times its probable error. This holds good for each kind of water although the difference is not very significant in the case of Jumna water.

*Other factors influencing the duration of life of the vibrios in the water*

Of the four experiments it will be seen that the earlier ones showed the duration of life to be much longer than that shown by the later experiments. The first and second experiments were started on the 21st February, 1928, and were finished on the 30th March, 1928 (including the last ten days in which none of the samples was positive). The second experiment is not strictly comparable with the first and the others, because in this experiment the vibrios added were  $1/10$ th the weight used in the other experiments. The third experiment was started on the 27th March, 1928, and was finished on the 26th April, 1928, and the fourth experiment started on the 3rd April and finished on the 20th April, 1928 (both including the last 10 days in which none of the samples was positive). As the fourth experiment ended during the time of the third experiment it will do if we compare the first with the third experiment only. The duration of life of the vibrios in the two experiments is as given below —

Vibrio	EXPERIMENT I		EXPERIMENT III	
	Boiled water	Unboiled water	Boiled water	Unboiled water
Inagglutinable	$15 \pm 96$ days	$80 \pm 42$ days	$94 \pm 23$ days	$10 \pm 63$ days
Agglutinable	$30 \pm 103$ days	$24 \pm 84$ days	$42 \pm 67$ days	$4 \pm 17$ day

Both kind of vibrios survived for a much longer period in the first than in the third experiment. The difference is significant taking into consideration the probable error involved. The inagglutinable vibrio lived certainly longer in the unboiled water in the first experiment than in the third, while in boiled water the difference is not significant. Whether this difference in the duration of life as given by the two experiments is due to the room temperature at which the flasks containing the water were kept or to the absolute humidity we have no sufficient data to tell. Suffice it to say that the mean temperature was  $73^{\circ}\text{F}$  during the time of the first experiment and  $86.0^{\circ}\text{F}$  during the time of the third experiment, and the mean absolute humidity 388 and 607 respectively. One can only say that in the period covered by these four experiments (21st February to 20th

# THE GERMICIDAL ACTION OF THE ACTIVATED SLUDGE PROCESS

BY

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WITH the introduction and development of the 'biological' processes for the treatment of sewage, it was hoped by sanitarians (on the analogy of the 'biological' action in water purification by slow sand filtration) that the effluents would be not only 'inoffensive,' but 'safe' These hopes were not realized and where effluents after treatment by septic tanks and bacterial beds were discharged into bodies of water to be subsequently used as sources of domestic water-supplies, it has been found necessary in many instances to sterilize the effluent, usually by some form of 'chlorination' On the banks of the Hooghly near Calcutta, where there are many installations of septic tank latrines and trickling filters, it has been found that effluents, though often chemically satisfactory, differ bacteriologically very little from raw sewage It is, therefore, probable that pathogenic organisms could run the gauntlet of such treatment without much diminution in number

The activated sludge process, the newest of the biological treatments, has the striking feature that it reduces the bacterial content of the sewage very considerably This fact has been drawn attention to by various writers Russel and Barlow(1) obtained 90 to 99 per cent reduction of the coli group after 4 hours' aeration Sierp and Hayo Bruns(2) got a similar reduction in 6 hours The senior writer also drew attention to this remarkable action in a paper read at the 7th Congress of the Far Eastern Association of Tropical Medicine, in Calcutta(3) Few observations have, however, been made on the bactericidal

*Result of the chemical analysis of the water samples*

Particulars	Ganges water *	Jumna water *	Well water *
Physical Characters	Highly turbid and suspended matter deposited at the bottom	Turbid and suspended matter deposited at the bottom	Slightly turbid and few suspended matter deposited at the bottom
Reaction	Slightly alkaline	Neutral	Very slightly alkaline
Free and Saline Ammonia Parts per 100,000	02	012	012
Albuminoid Ammonia Parts per 100,000	0274	011	005
Oxygen absorbed from Pot Permanganate in 3 hours at 37°C Parts per 100,000	16	084	13
Total Solids Parts per 100,000	20	24	68
Fixed Solids Parts per 100,000	12	18	36
Volatile Solids Parts per 100,000	8	6	32
Appearance on Ignition	Brown charring	No charring	Brown charring
Total Hardness Parts per 100,000	13	13	45
Temporary Hardness Parts per 100,000	8	10.6	30
Permanent Hardness Parts per 100,000	5	2.4	15
Chlorine Parts per 100,000	1	8	5.8
Nitrites	0015	001	Nil
Nitrates	Traces	Traces	045

\* The following were entered in the remarks columns of the original —

Ganges water—'Shows organic pollution'

Jumna water—'Free and saline ammonia slightly in excess, otherwise potable'

Well water—'Very high chlorine content, hard water, some organic pollution'

## REFERENCES

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- (3) KHAN, SARANJAM (1927) 'Cholera in Hardwar' Transactions of the Seventh Congress of the Far Eastern Association of Tropical Medicine, Vol II
- (4) PEARL, R (1923) 'Introduction to Medical Biometry and Statistics,' pp 258-259 W B Saunders Company, London

(2) *Selective bactericidal action on the different species of B coli*

Clemesha's classification was followed. Class I is the least resistant to unsuitable environmental conditions (e.g., exposure to light), and includes the ordinary *B coli communis*.

Class III is the most resistant to such influences and includes members like *B cloacæ*.

Class II is intermediate between Class I and II.

The sewage before and after aeration was plated on McConkey's bile salt lactose-agar, and twenty colonies from each sample examined by growing first in trypsin broth, and then inoculating into the various sugars. The results are shown in the table below —

*Percentage of classes of B coli present in aerated sewage after periods of aeration*

Class	AFTER AERATION OF							
	Raw	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	24 hrs
I	26.3 per cent.	17.2 per cent	13.4 per cent	12.2 per cent	12.1 per cent	11.8 per cent	10.5 per cent	10.3 per cent
II	62.2 „	65 „	67 „	62.6 „	59.5 „	58.2 „	51.5 „	48.7 „
III	11.5 „	17.8 „	19.5 „	25.2 „	28.4 „	30 „	38 „	41 „

From this it will be seen that there is a rapid reduction in Class I starting immediately, reaching a maximum after 6 hours' aeration, continued aeration for 24 hours does not reduce the proportion markedly, as regards Class II, the initial rise is apparent only, this class (and Class III) taking the place of the diminished Class I. After 2 hours' aeration, there is a distinct fall in the proportion of Class II organisms present, at the end of 6 hours, about half of the organisms still remaining belong to Class II, and farther aeration up to 24 hours does not affect the proportion materially.

In Class III, we note a proportional increase from the beginning of aeration, the percentage increasing progressively until at the end of 24 hours' aeration this class constitutes 41 per cent of the coli group remaining. During aeration, there is of course a large diminution of actual coliform organisms, the table given above merely denoting the proportion present after aeration. For practical purposes, it may be remembered there is a very marked reduction in the proportion of Class I organisms, a marked reduction of Class II and a marked increase in the proportion of Class III. A period of 6 hours' aeration may be taken for practical purposes as sufficient to ensure these changes. As the pathogenic intestinal organisms are grouped along with Class I, the sanitary benefit of the activated sludge process is at once evident.

The incubation, after shaking, was done in a water bath at 37°C Incubation in air gave identical results

The 'time consuming'(3) technique of the Wassermann test was not allowed to interfere with 'Kahn test in all its required details'(3) The two tests were done on different days

The antigen ready for use was kindly supplied by Dr Kahn himself Its titre was stated on the bottle The titre is now known to remain constant almost indefinitely(4, 5, 6)

The average was struck in accordance with the plan given in his book with the addition that  $\frac{+++++}{3}$  was taken as +++

## 2 *Microkahn*

A one tube test as recommended by Kahn in his book and in a subsequent contribution(7) was done Apparatus —

- i Small wooden racks 32 by 2 by 4 cm painted black and numbered to hold 16 of
- ii Durham's fermentation tubes
- iii Antigen dilution tube as prescribed for the regular test
- iv A 0.1 c.c. pipette divided into 0.01 c.c. A fermentation tube not admitting the pipette with perfect ease should be rejected
- v Wright's pipette calibrated with mercury to deliver 0.1 c.c.
- vi Wright's pipette calibrated with mercury to deliver 0.2 c.c.

*Procedure*—Two workers work together Worker No 1 deposits 0.01 c.c. of the antigen dilution at the bottom of the tubes The rack when finished is handed over to worker No 2 who, with the calibrated Wright's pipette, delivers 0.1 c.c. of the serum to be tested into each tube Worker No 2 is assisted by two assistants Assistant No 1 hands him the numbered serum in a suitable container, assistant No 2 hands him a clean 0.1 c.c. calibrated Wright's pipette He also picks up the used pipette, washes it out twice with water and once with saline and removes excess of saline on a filter paper

The racks are shaken vigorously for two minutes and placed in the incubator

Fifteen minutes later the racks are removed from the incubator and 0.2 c.c. saline added with the calibrated Wright's pipette to each tube

### *The ensemble*

Antigen dilution	0.01 c.c.
Serum	0.1 c.c.
Shaken for two minutes	
Incubated for fifteen minutes	
Saline	0.2 c.c.

Results are read by holding the rack in front of a window (refracted light) aided by holding the index finger between the light and the tube, by the lower edge of the rack (reflected light)

# ON THE DURATION OF THE LIFE OF VIBRIOS IN THE GANGES AND JUMNA RIVER WATER

BY

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THE rivers Ganges and Jumna are two very important rivers of Hindustan and in the Hindu religion both possess special sanctity. A large number of fairs take place every year on the banks of these rivers and the object of the pilgrims attending them is to bathe, especially in the Ganges. When bathing all pilgrims drink the river water and many take some of the water to their homes. This water is offered to images or is given in small quantities to the dying, or is consumed by those who did not make the pilgrimage.

It is common knowledge that these annual fairs play a very important part in the spread of cholera. It is found that at the time of these gatherings, the river water receives a high degree of pollution from night-soil, bathing and washing. The intensity and virulence of the pollution depend upon many things such as the nature of the locality, the quantity of water, the magnitude of the gathering and the time of the year. At Hardwar there is evidence to show that the polluted water of the Ganges is responsible for the spread of cholera there and its importation into other provinces.

Hankin (1896) (1) concluded that cholera vibrios added to water from the Jumna river disappeared entirely within 4 hours. According to him the water of certain rivers of India possesses an extremely marked bactericidal action in general and for cholera vibrios in particular. Hankin states that the water of the Jumna, as it left the town of Agra, contained more than 100,000 bacteria per cubic centimetre, while some 5 kilometres further down, the bacterial count was reduced to between 90 and 100 organisms.

Dealing more particularly with the cholera vibrio, Hankin's laboratory findings gave the results presented in the following table. He inoculated water from the Jumna river with a culture of *Vibrio cholerae* and the rate of action was shown by the bacterial counts made after different intervals.



The results are read as —Double Positive ++, Positive +, Doubtful ±, Irregular—When both the tubes in the test proper show partial lysis Negative—

The irregular reactions appear to have the same history as doubtful reactions In the present work the two reactions have been shown separately and also together

A partial inhibition of lysis in tube No 3 is matched against a similar inhibition, if present, in tube No 1 and disregarded if equal or almost equal This inhibition is brought about by the anti-complementary titre of the serum lying between  $2\frac{1}{2}$  and 1 M H D

## II COMPARISON OF THE RESULTS OF 1,000 SERA BY WASSERMANN, KAHN AND MICROKAHN TESTS

The sera tested were from unselected cases and were received from all over Burma, with short clinical histories The results obtained are shown in Table I in which the findings by Kahn and Microkahn tests are contrasted with the Wassermann results

The Wassermann and Kahn tests showed an absolute agreement in 69.6 per cent of sera, a relative agreement in 23.4 per cent and no agreement in 7 per cent The corresponding figures for the Microkahn were 66.3 per cent, 26.1 per cent and 7.6 per cent respectively

These figures may be compared with those of HULL(9) and KAHN, KENDRICK and LANDAU(10) in the following summary —

Author	Number of sera	Percentage absolute agreement	Percentage relative agreement
Hull	26,000	94.9	97.8
Kahn, Kendrick and Landau	300,000	96.7	99.3
Greval	1,000	69.6	93

KILDUFFE(11) in comparing the Wassermann and the Microkahn tests obtains a 'total relative agreement' of — 63 per cent 'if the plus-minus and plus micro-reaction is regarded as without significance and thus in accord with a negative Wassermann' (absolute agreement—writer) " 90 per cent 'if on the other hand, the plus-minus and plus one micro-reaction is awarded significance and is thus in agreement with a positive Wassermann reaction' (relative agreement—writer)

## PROTOCOL

	Source of water	QUANTITY	
		Water	Inagglutinable vibrio
Ganges water	Raw water	100 cc	0.111 mg
	Raw but filtered	100 cc	0.111 mg
	Boiled	100 cc	0.111 mg
	Boiled and filtered	100 cc	0.111 mg
Jumna water	Raw water	100 cc	0.111 mg
	Raw but filtered	100 cc	0.111 mg
	Boiled	100 cc.	0.111 mg
	Boiled and filtered	100 cc	0.111 mg
Sangam	Raw water	100 cc	0.111 mg
	Raw but filtered	100 cc	0.111 mg
	Boiled	100 cc	0.111 mg
	Boiled and filtered	100 cc	0.111 mg
Mixture, e.g., equal quantities of Ganges and Jumna water	Raw water	100 cc	0.111 mg
	Raw but filtered	100 cc	0.111 mg
	Boiled	100 cc	0.111 mg
	Boiled and filtered	100 cc	0.111 mg
Well water	Raw water	100 cc	0.111 mg
	Raw but filtered	100 cc	0.111 mg
	Boiled	100 cc	0.111 mg
	Boiled and filtered	100 cc	0.111 mg
	Distilled water	100 cc	0.111 mg

A similar protocol was used for the agglutinable vibrio. If vibrios could not be grown from the water under the conditions stated above, it was presumed that their life had become extinct. We then had to fix the number of days the sample should be free from viable vibrios under the conditions stated above to enable us to conclude, with reasonable certainty, that the results are not due to chance and that the water really had become free from

Once again the Kahn and Microkahn tests bring out practically the same number of positive cases. These cases, however, are not identical (*see* Table II)

#### IV ASSOCIATED CONSIDERATIONS AND COMMENTS

##### 1 *Kahn*

- i Irregular reactions In the three tube test considerable irregularity of reaction was encountered. Reactions under 'four plus' instead of increasing with the decreasing quantity of the antigen dilution either decreased or remained constant. 'Four plus' reactions decreased with the decrease in the antigen dilution. In the series of 1,000 cases, out of 382 positive cases, only 156 were regular.
- ii Increased turbidity Contents of certain tubes while free from any granularity were found to be definitely more opalescent than the serum or the antigen dilution. These sera did not produce milkiness on addition of saline.

##### KAHN

Out of a series of 775 cases, 11 cases showed increased turbidity

W R, positive	4
W R, doubtful	3
W R, irregular	1
W (R), negative	3
	—
	11
	—

##### MICROKAHN

Out of a series of 775 cases, 10 cases

showed increased turbidity	
W R, positive	3
W R, doubtful	2
W R, irregular	2
W R, negative	3
	—
	10
	—

- iii Delayed reactions In a long series results were re-read after 24 hours. Some sera were found to have turned positive from negative or more positive than on the previous day. The change affected both Wassermann positive and Wassermann negative sera. The majority of the sera, however, remained negative.
- iv Flakes In certain sera, in addition, a precipitate flake formation occurred. In others the same flakes were to be seen in otherwise negative tubes. These flakes were disregarded. They appeared to be unconnected with the physical quality of the sera. All the sera were free from particles.
- v Quantitative procedure 'The procedure is applicable only to those cases which give four plus reaction with the routine test' (12). Of such cases out of 382 positive cases, there were only 40 in the series of 1,000 cases. The procedure could thus be used in about one case out of 9 positive cases.

that all vibrios had died off. Now in the first experiment the samples were taken on alternate days and the last positive sample was on the 26th day. From the 42 flasks there were taken 546 samples in 26 days, of which 116 were positive. The standard deviation is 9.55. Now after the last day any sample was positive there were taken 420 samples in 10 successive days. If during these 10 days the conditions influencing the life of vibrios were exactly the same as in the preceding 26 days one would expect 89.22 positive samples. Actually none of the 420 samples was positive. The difference is therefore 89.22 and this is 9.37 times the standard deviation. Now the chances are nil for such a great difference to occur as to be 9 times the standard deviation. This experiment was repeated 4 times in the course of 3 months with similar results, thus in the second experiment the difference is about 8 times the standard deviation, and in the third and fourth experiments 6 and 10 times respectively. In other words if living vibrios were present in any one of the 42 flasks, it is mathematically certain that we would not have failed to find them in samples taken daily for 10 successive days.

The results of the four experiments are given in Tables I to IV. From these tables it will be observed that there are some figures which are obviously due to some error in technique. For instance in experiment No III in Jumna unboiled filtered water (magglutinable vibrio), the vibrio was found only once in 15 samples, i.e., on the 20th day. This was not likely to occur because the percentage of positive cases in the total number of samples was much higher. On the average 64 per cent of the samples were found positive, thus in a random sample of 15 there will not be found fewer than 6 positive samples as often as once in 200 trials. Odds of 199 to 1 are sufficiently wide to constitute certainty in most practical statistical matters, Pearl (1923) (4).

For the total of the four experiments the mean percentage of positive samples comes to  $64 \pm 1.91$  and the standard deviation to 6.27. For any flask, therefore, if the samples are 25 per cent or less than 25 per cent positive it is presumed that such a low find is significant and cannot be due to chance alone taking into consideration the probable error involved. In other words in case of flasks of which 25 or less samples in a 100 were positive, it was presumed that probably there were no viable vibrios present and that the occasional find was due to some mistake. Making allowance for this correction we will have the results as set forth in the tables below.

### *The Effect of Filtration*

The duration of life of vibrios in filtered as compared with unfiltered water is given in Table V. It will be seen that mere filtration of the water through filter paper has no influence on the duration of life of either the agglutinable or the magglutinable vibrio. This holds good individually for each experiment and also for the average of all the four experiments. The kind of water and the temperature of the room make no difference. Thus the average duration of life of the magglutinable vibrio for the aggregate of all the four

The following is an abstract from the history of one of them —

Mr X, a final year medical student. Doubt as to having contracted syphilis W R, negative in October 1925, July 1926 and February 1927 Seen by three medical men

*The ensemble*

	1st tube 1 in 2	2nd tube 1 in 4	3rd tube 1 in 8	4th tube 1 in 16	5th tube 1 in 32	6th tube 1 in 64
Serum 1 Vol of —						
Wassermann antigen, 1 in 6						
saline mixture in Vol —	1	1	1	1	1	1

Incubated at 37.5° for 4 hours

Left at room temperature overnight.

Results — Definite flocculation in 1st and 2nd tube only, weak positive Weaker reaction, doubtful

Complaint since 1925 Cervical, epitrochlear and inguinal glands enlarged and shotty Catarrh of nasopharynx Frequent stomatitis Shifting pains Loss of weight A lymphocytosis present (42 per cent lymphocytes) Marriage in view, hence the anxiety All other probable conditions excluded The writer believed there was an indication for further observation and therapeutic tests

SUMMARY

- 1 The Kahn test is not so sensitive as the Wassermann test
- 2 The Kahn test is not so specific as the Wassermann test
- 3 The Kahn test alone will miss more cases than the Wassermann test alone
- 4 The Kahn test alone will return more false positive results than Wassermann test alone
- 5 In conjunction with the Wassermann test, the Kahn test is useful in detecting certain cases of syphilis either missed or returned as doubtful by the Wassermann
- 6 For use in conjunction with the Wassermann, the Microkahn test is as good as the Kahn
- 7 A suspected case of syphilis may be completely missed by both Wassermann and Kahn tests and be detected by the Sachs-Georgi test

TABLE I

*Giving the results of 1,000 sera tested by the Wassermann, Kahn and Microkahn tests*

Wassermann		KAHN			MICROKAHN		
		Positive* (++++, +++ or ++)	Doubtful* (+ or ±)	Negative	Positive	Doubtful	Negative
Positive	425	323	59	43	313	66	46
Doubtful	165	30	36	99	39	36	90
Doubtful, proper	53	11	9	33	14	11	28
Irregular	112	19	27	36	25	25	62
Negative	165	30	36	99	39	36	90
	410	27	46	337	30	66	314
TOTAL	1,000	380	141	479	382	168	450

\* As recommended by Kahn (9)

TABLE II  
Duration of life of the maggotuntable vibrio in the water given below

Source of water	EXPERIMENT I				EXPERIMENT II				EXPERIMENT III			EXPERIMENT IV		
	Last day of positive samples, i.e., duration of life	Number of positive samples in the total	Percentage of positive samples		Last day of positive samples, i.e., duration of life	Number of positive samples in the total	Percentage of positive samples		Last day of positive samples, i.e., duration of life	Number of positive samples in the total	Percentage of positive samples	Last day of positive samples, i.e., duration of life	Number of positive samples in the total	Percentage of positive samples
Ganges	8	2 in 4	50.0		14	7 in 7	100.0		21	2 in 16	12.50	0	0	0
	8	4 in 4	100.0		20	6 in 10	60.0		21	3 in 16	18.75	8	3 in 7	42.85
	10	4 in 5	80.0		14	4 in 7	57.4		9	3 in 4	75.0	6	4 in 5	80.0
	12	5 in 6	83.3		10	2 in 5	40.0		10	4 in 5	80.0	2	1 in 1	100.0
Jumna	8	3 in 4	75.0		20	3 in 10	30.0		2	1 in 1	100.0	0	0	0
	8	3 in 4	75.0		8	2 in 4	50.0		20	1 in 15	6.6	0	0	0
	12	6 in 6	100.0		18	7 in 9	77.7		10	3 in 5	60.0	4	2 in 3	66.6
	14	5 in 7	71.4		14	7 in 7	100.0		11	4 in 6	66.6	4	1 in 3	33.3
Sangam	8	3 in 4	75.0		10	3 in 5	60.0		2	1 in 1	100.0	3	2 in 2	100.0
	10	3 in 5	60.0		14	4 in 7	57.1		2	1 in 1	100.0	3	2 in 2	100.0
	18	7 in 9	77.7		18	6 in 9	66.6		10	5 in 5	100.0	4	3 in 3	100.0
	16	6 in 8	75.0		20	8 in 10	80.0		7	1 in 2	50.0	0	0	0
Mixture	8	4 in 4	100.0		0	0	0		0	0	0	3	2 in 2	100.0
	4	2 in 2	100.0		10	1 in 5	20.0		21	2 in 16	12.5	0	0	0
	12	5 in 6	83.3		0	0	0		10	5 in 5	100.0	4	3 in 3	100.0
	12	4 in 6	66.6		2	1 in 1	100.0		8	3 in 3	100.0	4	3 in 3	100.0
Well	12	4 in 6	66.6		0	0	0		2	1 in 1	100.0	4	2 in 3	66.6
	6	3 in 3	100.0		29	1 in 15	6.6		2	1 in 1	100.0	3	1 in 2	50.0
	18	8 in 9	88.8		0	1 in 5	20.0		10	4 in 5	80.0	3	2 in 2	100.0
	26	7 in 13	53.8		10	0	0		9	4 in 4	100.0	4	3 in 3	100.0
Distilled water	0	0	0		0	0	0		0	0	0	0	0	0

- (4) CORRESPONDENCE (1927) *Jour Amer Med Assoc*, May 21st, p 1663
- (5) KENDRICK and JEANS (1926) *Jour Lab and Clin Med*, January
- (6) KAHN, R. L., NAGLE N and KENDRICK, R. L (1927) *Jour Inf Dis*, August
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- (10) KAHN, R. L, KENDRICK, P L, and LANDAU, T L (1927) *Ibid*, July 9th, p 84
- (11) KILDUFFE, R A (1927) *Jour Lab and Clin Med*, July
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- (13) *Idem* (1925) p 181
- (14) KAHN, R. L, NAGLE, N and KENDRICK, P L (1927) *Jour Inf Dis*, August
- (15) MACKIE and MCCARTNEY (1925) 'An Introduction to Practical Bacteriology' Edinburgh, E and S Livingstone
- (16) KAHN, R L (1925) p 168

TABLE IV  
Duration of life of the maggotinable vibrio in water given below Duration of life in waters of which the percentage of positive samples is as low as 25 per cent or less has been taken as zero

Source of water	EXPERIMENT I				EXPERIMENT II				EXPERIMENT III			EXPERIMENT IV			
	Last day of positive samples, i.e., duration of life.	Number of positive samples in the total	Number of samples	Percentage of positive samples	Last day of positive samples, i.e., duration of life.	Number of positive samples in the total	Number of samples	Percentage of positive samples	Last day of positive samples, i.e., duration of life.	Number of positive samples in the total	Number of samples	Percentage of positive samples	Last day of positive samples, i.e., duration of life.	Number of positive samples in the total	Percentage of positive samples
Ganges	8	2 in 4	4	50.0	14	7 in 7	7	100.0	0	0	0	0	0	0	0
	8	4 in 4	4	100.0	20	6 in 10	6	60.0	0	0	0	0	8	3 in 7	42.85
	10	4 in 5	4	80.0	14	4 in 7	4	57.4	9	3 in 4	75.0	80.0	6	4 in 5	80.0
	12	5 in 6	5	83.3	10	2 in 5	2	40.0	10	4 in 5	80.0	100.0	2	1 in 1	100.0
Jumna	8	3 in 4	3	75.0	20	3 in 10	3	30.0*	2	1 in 1	100.0	100.0	0	0	0
	8	3 in 4	3	75.0	8	2 in 4	2	50.0	0	0	0	0	0	0	0
	12	6 in 6	6	100.0	18	7 in 9	7	77.7	10	3 in 5	60.0	60.0	4	2 in 3	66.6
	14	5 in 7	5	71.4	14	7 in 7	7	100.0	11	4 in 6	66.6	66.6	4	1 in 3	33.3
Sungam	8	3 in 4	3	75.0	10	3 in 5	3	60.0	2	1 in 1	100.0	100.0	3	2 in 2	100.0
	10	3 in 5	3	60.0	14	4 in 7	4	57.1	2	1 in 1	100.0	100.0	3	2 in 2	100.0
	18	7 in 9	7	77.7	18	6 in 9	6	66.6	10	5 in 5	100.0	100.0	4	3 in 3	100.0
	16	6 in 8	6	75.0	20	8 in 10	8	80.0	7	1 in 2	50.0	50.0	0	0	0
Mixture	8	4 in 4	4	100.0	0	0	0	0	0	0	0	0	3	2 in 2	100.0
	4	2 in 2	2	100.0	0	0	0	0	0	0	0	0	0	0	0
	12	5 in 6	5	83.3	0	0	0	0	10	5 in 5	100.0	100.0	4	3 in 3	100.0
	12	4 in 6	4	66.6	2	1 in 1	1	100.0	8	3 in 3	100.0	100.0	4	3 in 3	100.0
Well	12	4 in 6	4	66.6	0	0	0	0	2	1 in 1	100.0	100.0	4	2 in 3	66.6
	6	3 in 3	3	100.0	0	0	0	0	2	1 in 1	100.0	100.0	3	1 in 2	50.0
	18	8 in 9	8	88.8	0	0	0	0	10	4 in 5	80.0	80.0	3	2 in 2	100.0
	26	7 in 13	7	53.8	0	0	0	0	9	4 in 4	100.0	100.0	4	3 in 3	100.0
Distilled water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

\* For calculating the mean, etc., this although a little above 25 is also taken as zero owing to the extraordinary long duration of life



are normally found in such an essentially lymphoid organ as the appendix. A diagnosis based on measurements of the submucosa is also open to the fallacy that such measurements depend upon many factors as, for example, on the state of the lumen. If the lumen is dilated, the coats are all likely to be stretched out and a thickened submucosa may appear even thinner than normal. Then again it is quite common to find an area of thickening in the submucosa on one side in a normal appendix opposite the mesenteric attachment. The presence of peri-vascular infiltrations are not of much moment since lymphoid tissue is quite usual in the appendix and such peri-vascular infiltrations may be found in normal appendices removed from the post-mortem room in which there are no obvious traces of inflammatory reaction.

The following table shows the number of normal appendices in which peri-vascular infiltrations were found as well as the number of chronic appendices in which they were in evidence —

*Peri-vascular infiltrations in the submucosa of the appendix*

Number of diseased appendices examined 173	Number in which peri-vascular infiltrations were present 48	Percentage 27.8
Number of normal appendices examined 236	Number in which peri-vascular infiltrations were present 36	Percentage 15.3

The percentage of cases of normal as well as abnormal appendices in which infiltration of the mucosa with lymphoid cells was present is given as follows —

*Lymphoid accumulations in the submucosa*

Number of diseased appendices examined 173	Number in which lymphoid infiltrations were present 173	Percentage 100
Number of normal appendices examined 236	Number in which lymphoid infiltrations were present 232	Percentage 98.3

With regard to infiltrations with plasma cells, these are exceedingly difficult to find in the appendix. They, however, occur in greater frequency in chronically inflamed appendices as shown below —

*Plasma-celled infiltrations of the submucosa*

Number of diseased appendices examined 173	Number in which plasma-celled infiltrations were present 109	Percentage 63.0
Number of normal appendices examined 236	Number in which plasma-celled infiltrations were present 59	Percentage 25.0

experiments comes to  $70 \pm 68$  days in filtered and  $685 \pm 61$  days in unfiltered water. The difference is  $17 \pm 9$  which is less than its probable error and is therefore certainly not significant\*. The same holds good for the agglutinable vibrio (Difference in means =  $6 \pm 7$ )

### *The Effect of Boiling (5 minutes)*

The results showing the duration of life of the vibrios in boiled and unboiled water are given in the table below (Table VI). Boiling the water for 5 minutes makes it more suitable for the vibrios to live, that is to say, the vibrios live longer in boiled than in unboiled water. There appears to be some substance in unboiled water which shortens the life of vibrios and this substance is destroyed or is appreciably diminished by simply boiling the water for 5 minutes. Based on the results of all the four experiments the average duration of life of the inagglutinable vibrio in unboiled water comes to  $45 \pm 52$  days and in boiled water to  $937 \pm 65$  days. The difference is  $487 \pm 83$  which is more than 5 times its probable error. It is, therefore, certainly significant.

Like the inagglutinable vibrio, the agglutinable vibrio also lived longer in boiled than in unboiled water. It survived on the average for  $45 \pm 58$  days in boiled and  $11 \pm 29$  days in unboiled water. This is certainly significant as the difference is more than 5 times its probable error (Difference =  $34 \pm 65$ ).

Thus thermolabile vibriocidal substance is present to a more or less extent in all the three kinds of water used in this experiment. It is of the highest potency in the Jumna water, of somewhat lesser potency in the well water and of the least potency in the Ganges water (*Magh Mela* site, Allahabad). The difference of the duration of life of the inagglutinable vibrio in boiled and unboiled water comes to  $762 \pm 14$  days in Jumna water,  $513 \pm 22$  days in well water, and  $187 \pm 18$  days in Ganges water. The corresponding figures for the agglutinable vibrio are  $6 \pm 206$  days in Jumna,  $237 \pm 97$  in well, and  $1 \pm 77$  days in Ganges water.

As an explanation of the above we quote the opinion of Lieut-Col C. I. Dunn, CIE, DPH, IMS, Director of Public Health, United Provinces, who writes as follows on this question: 'The reason for the longer life of both kinds of vibrios in boiled water is obviously due to the fact that by boiling water the bacteria of all kinds are killed, so that when cholera vibrios are added, they have, therefore, the dead bodies of these bacteria to feed on. I should think they live just as long as there is a food supply for them to live on. This cannot be long, because the vibrios will multiply to begin with and die off as the food is exhausted.'

'This is obviously the reason why the duration of life in distilled water is so short, as in distilled water there is no food supply whatever.'

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\* It is generally accepted that a difference to be of any significance must be 3 or more times its probable error.

The presence of marked fibrosis and thickening of the submucosa are, however, indicative of inflammatory change, but here again measurements are not of much value since the thickness depends to a great extent on the size of the lumen

I have in a previous paper referred to marked infiltration of the submucosa with eosinophile leucocytes in the chronic appendix. They occur in 88.6 per cent of cases of chronic appendicitis. They are not obvious in the normal appendix except in between the cells of the mucous membrane and their presence in the submucous tissue in the appendix is of great value for histological diagnosis

Changes in the muscle coat in chronic appendicitis vary from atrophy of the muscle coat which occurs in cases of concretions. Hypertrophy of the muscle coat has been met with in a few cases but the significance of this is not quite clear. Fibrosis of the muscle coat or infiltration of the muscle coat with round cells or eosinophile cells are of great histological value as they obviously indicate previous or recent inflammatory changes

Marked thickening of the serous coat with cell infiltrations may be met with in subacute appendicitis but are not obvious in the chronic disease. This again raises the theory of the mode of spread of infection from the mucosa and submucosa through the *Hiatus muscularis* to the serous coat resulting in localized peritonitis which is so common. Congestion of the vessels of the serous coat is met with in congested appendices without obvious inflammatory signs. Such changes seem to depend entirely on the state of the vessels

#### SUMMARY

(1) In the histological diagnosis of chronic appendicitis the three features that are of importance are the presence of marked fibrosis and thickening of the submucosa, eosinophile infiltrations of the submucosa and fibrosis or cell infiltrations of the muscle coat

(2) Each of these by itself is sufficient to warrant a report of chronic appendicitis

'The shorter duration of life in unboiled water may be due to the presence in that water of other bacteria inimical to the cholera vibrios or merely competitors for the available food supply. If this is so, it would explain the shorter duration of life. It may, therefore, have nothing to do with a thermolabile vibriocidal substance at all. The argument against this is that if this were so, the vibrios would die off in raw water much quicker than they would in filtered unboiled water, whereas there appears to have been very little difference in this particular.'

*The duration of the life of vibrios in each kind of water*

The duration of the life of vibrios in each kind of water varied and is shown in Table VI. In the case of boiled water the magglutinable vibrios survived for the number of days given below —

In Jumna water	10.87 ± 1.08 days
In Ganges water	9.12 ± .81 „
In well water	8.75 ± 2.05 „

The difference in the duration of life in each water, as given above, is insignificant taking into consideration the probable error involved.

In unboiled water the vibrios survived for the number of days given below —

In Jumna water	3.25 ± .88 days
In well water	3.62 ± .87 „
In Ganges water	7.25 ± 1.61 „

The difference in the duration of life of the magglutinable vibrios in unboiled Jumna water as compared with unboiled well water is insignificant whereas in case of both Jumna and well water when each is compared with the Ganges water the difference is significant taking into consideration the probable error involved.

Thus the vibrios survived for a considerably longer period in the Ganges water as compared with either the Jumna or the well water. This difference is only marked in raw water, because when the water is boiled the duration of life becomes equal in all the three kinds of water. In other words Jumna water or well water at Allahabad is less suitable to the life of vibrios than the Ganges water and that this property is destroyed by boiling the water. Similar results were obtained in the case of the agglutinable vibrio, the duration of life being —

	Boiled	Unboiled
In Jumna water	7 ± 1.97 days	1 ± .62 days
In well water	3.37 ± .86 „	1 ± .47 „
In Ganges water	2.0 ± .46 „	1 ± .62 „

Under all circumstances the magglutinable vibrio lived much longer than the agglutinable vibrio. In boiled water the former lived for  $9.37 \pm .65$  days and

The preparations from the peripheral blood taken usually between the hours of 7 and 10 p m were stained by Giemsa or Hæmatoxylin on the approved lines and camera-lucida drawings of the parasites were made

I am indebted to my staff and particularly to Captain K K Das, my assistant, who brought the prevalence of the atypical forms to my notice

## RESULTS OBTAINED IN FIELD STUDIES

### *A Morphology of Atypical and Typical Forms*

*Typical Forms*—The leading anatomical characters of the embryos of *bancrofti* have been described previously (Korke, 1927 Preliminary Investigation)

Mean variation in length, 18 specimens, 212—323 M

Mean variation in breadth, 10 specimens, 4.5—6.5 M

For the purposes of comparison, I give here the mean and percentage measurements of *bancrofti* in microns from Balasore, Cuttack and Puri (Orissa area), and Gaya (Bihar area) Measurements of those anatomical parts are given here, which have a direct bearing on the subject-matter

	C S	N	Ex P	A P	L S Z	Length
BALASORE—						
Mean (4 specimens)	6	45	64	170	205	212
Percentage		21	30	80	97	
CUTTACK—						
Mean (5)	8	57	87	244	276	288
Percentage		20	30	85	96	
PURI—						
Mean (4)	8	61	99	260	298	310
Percentage		20	32	84	96	
GAYA—						
Mean (5)	10	64	94	270	306	323
Percentage		20	29	84	95	

April, 1928), as the temperature and the absolute humidity rose the duration of the life of the vibrios became shorter

### GENERAL REMARKS

As has been said the agglutinable vibrio used in the experiments was isolated from a case of cholera in Hardwar in 1927. It agglutinated with the standard cholera serum to a dilution of 1:8,000 (full titre). It showed —

- 1 Typical colonies
- 2 Good vibronic morphology
- 3 Active motility
- 4 General turbidity and pellicle formation in peptone water
- 5 Liquefaction of serum (typical)
- 6 Formation of acid but no gas in glucose and saccharose (24 hours)
- 7 Growth in milk without acid formation or clotting (24 hours)
- 8 Cholera red reaction—well marked

The inagglutinable vibrio showed all the above characteristics, only it did not agglutinate with the standard serum even in a dilution of 1:80.

The agglutinable vibrio was a thin slender curved rod with rather a long curve, while the inagglutinable one was a thick short vibrio with a sharp curve. Although towards the end of their life granular and degenerate forms appeared in the case of both kind of vibrios, yet the original characteristic morphology was retained to the last.

During the entire period of these experiments we came across only three instances in which an inagglutinable vibrio was isolated from the flasks containing agglutinable vibrios. In every one of these three instances the vibrio was found only once on the last day after a long interval of negative days. Such an occasional find, after a large number of negative days, we regarded to be probably due to some mistake.

The water was also examined chemically by Rai Bahadur Dr D D Pandya, DPH (Camb), etc, Assistant Director of Public Health (Provincial Hygiene Institute), U P, Lucknow, and the results are given in the table below.

In distilled water the vibrios died-out very soon. They could not be isolated from samples taken about 20 hours after they were added to the water.

We are very thankful for the valuable guidance and advice given by Lieut-Col C L Dunn, CIE, DPH, IMS, Director of Public Health, United Provinces, who directed the Research and to Rai Bahadur Dr D D Pandya, DPH (Camb), etc, Assistant Director of Public Health (Provincial Hygiene Institute), U P, under whose supervision the Research Work was conducted in the United Provinces.

On the whole, the atypical forms appear to be shorter and less wavy, are filled with coarse coalescing granules, differ in measurements and show characters of anal pore and tail, which I think would be sufficient grounds to classify them as separate forms from the type species of *bancrofti*

*B Incidence of Atypical and Typical Forms, (see Table I)*

Out of 106 persons examined at Jalleswar, 24 showed atypical forms in the peripheral blood while typical forms were found to be negative

Out of 117 persons examined at Soro, 15 showed atypical forms and none typical

Out of 108 persons examined at Basudebpur, 28 showed atypical forms and 3 typical

Out of 467 persons examined in the Balasore town area, 47 showed atypical forms and 29 typical

Out of 42 persons examined at Chandipur, 5 showed atypical forms and 6 typical

Out of 126 persons examined during the day time, at Bhadrak, none showed atypical forms and 5 showed typical

Out of 484 persons examined at Bhadrak during the night time, 2 showed atypical forms and 70 typical forms

Thus out of 161 per cent of the population which showed microfilaria in the peripheral blood, 78 per cent showed typical forms and 83 the atypical forms, or a little over half the number of persons who showed microfilaria in the peripheral blood

*C Distribution of Atypical and Typical Forms in Association with Elephantiasis and Affections of Tunica Vaginalis*

The predominant pathological expression in association with filariasis in the Bihar and Orissa area is the affection of the genitals, especially in the form of hydrocele and elephantiasis of the extremities, chiefly the lower

For the purpose of this paper, I define elephantiasis as a pathological condition which in association with filariasis results in a permanent oedema of some part of the body

The distribution of the parasites and the elephantoid and hydrocele conditions are of the following nature in the Balasore district area (Table I)

The following inferences can be drawn from this table —

(1) That cases of elephantiasis appear to be of frequent occurrence in an area where there is more prevalence of atypical forms, e g, Jalleswar and Soro

(2) That cases of hydrocele appear to be of frequent occurrence in an area where there is more prevalence of typical forms, e g, Bhadrak

(3) That in an area where both the forms begin to show in some numbers, cases showing affections of tunica vaginalis and oedema of extremities appear more or less in even numbers, e g, Basudebpur and Balasore town

# KAHN, MICROKAHN AND WASSERMANN TESTS A COMPARISON IN 2,066 CASES

BY

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THE introduction of flocculation tests such as the Kahn test for the serum diagnosis of syphilis has provided a method which should prove of great use in many parts of India where the facilities for the Wassermann test are not quickly available. The simplicity of the reagents and technique of the Kahn method should make it possible to carry out this test in hospital laboratories without much difficulty.

Numerous papers have been published on the comparative results of the Kahn and Wassermann tests as performed by different workers. High percentage agreement has as a rule been obtained and claims made for the greater reliability of one or the other method and correspondence with the clinical findings. Individual workers who have been accustomed to a particular technique and have by personal experience found its value naturally desire to make their own comparisons of the two methods.

The comparisons in the present paper have been instituted with the Wassermann test as carried out at the Pasteur Institute of Burma.

## I TECHNIQUE OF THE TESTS

### 1 *Kahn*

The technique as given by Kahn in his book(1) was adhered to except in the matter of dimensions of the tube used in the test. A tube 5 cm in length and 0.8 cm in diameter was used instead of a tube 7.5 cm in length and 1 cm in diameter. The small deviation was considered to fall well within the 'considerable flexibility'(2) permitted by the test.



TABLE II

*Showing Relativity of Atypical and Typical Forms in the Peripheral Blood*

Area	Total slides (cases)	Total parasites	Variations	Average	FORMS		Cases showing combined forms
					Atypical	Typical	
Jalleswar	11	374	4-196	34	All	None	None
Soro	9	495	2-214	55	All	None	None
Basudebpur	10	302	1-103	30	All	None	None
Do	1	90			88	2	1
Do	1	19			18	1	1
Balasore town	15	553	1-149	37	All	None	None
Do	1	5			4	1	1
Do	4	187	13-109	47	155	32	4
Bhadrak	2	9	3-6	5	All	None	None
Do	20	416	2-57	20	None	All	None

(3) That 10 per cent of cases showed both the forms, the average count per slide being 38 in the case of atypical and 5 in the case of typical

In other words, there was a preponderance of the atypical forms over the typical ones and the parasites tended to appear in the peripheral blood as representative of one type only

### *I: Relation of Atypical and Typical Forms with the Affections of the Genitals and Elephantiasis*

In this connection I give observations on cases which showed one affection only and in which the parasites belonging to one or other form were found

#### Typical Forms (Korke, 1929)

24 cases from 10 different areas in the Gaya and Patna districts (Bihar area) showed affections of tunica vaginalis in which typical forms were observed

5 cases from Bhadrak and 9 from Puri (Orissa area) showed affections of tunica vaginalis in which typical forms were observed

One case from Bihar area and two from Balasore area showed affection of the extremities in which typical forms were observed

#### Atypical Forms

One case from Basudebpur showed affection of tunica vaginalis in association with atypical form

0.7 cc of the antigen (minimum recommended by Kahn) diluted with the necessary quantity of saline (as indicated by the titre) suffices for about 100 tests which can be easily managed in one batch (i.e., the antigen dilution and the serum can be mixed within 20 minutes)

### 3 Wassermann

The method followed is essentially No. IV of the British Medical Research Committee (8) with the following differences —

- i A slightly larger amount of saline is used to increase the total volume and to facilitate the measurement of serum which is dropped from a Wright's pipette with the point fitting hole No. 58 of the standard wire gauge. Such a drop measures 0.02 cc.
- ii A smaller dose of complement is used, 4 M.H.D. and  $2\frac{1}{2}$  M.H.D. instead of 5 M.H.D. and 3 M.H.D.
- iii An additional serum control is put up containing the same functioning dose of the complement (deducting the dose destroyed by the antigen) as in the tube with the lower dose of the complement in the test. This control guards against a false positive reaction resulting from the anti-complementary titre of the serum lying between  $2\frac{1}{2}$  and 1 M.H.D.
- iv Lately, complement and antigen have been mixed immediately before use and added in one measurement.
- v The final incubation, after adding the sensitized red blood-cells, is done for  $\frac{1}{2}$  hour.

#### The ensemble

	Serum controls		Test proper	
	1st tube	2nd tube	3rd tube	4th tube
Serum	0.02 cc	0.02 cc	0.02 cc	0.02 cc
Saline	0.3 cc	0.3 cc	0.2 cc	0.2 cc
Complement Sol				
0.1 cc containing	$1\frac{1}{2}$ M.H.D.	$2\frac{1}{2}$ M.H.D.	$2\frac{1}{2}$ M.H.D.	4 M.H.D.
Antigen mixture	Nil	Nil	0.1 cc	0.1 cc
	Left at room temperature for $\frac{1}{2}$ hour			
	Incubated in water bath for $\frac{1}{2}$ hour			
Suspension of R. B. C. (3 per cent) sensitized (with 5 M.H.D. of hæmolytic amoceptor)				
	0.1 cc	0.1 cc	0.1 cc	0.1 cc
	Incubated in water bath for $\frac{1}{4}$ hour			

Following the above line of argument as a guide, it appears to me that the forms are distinct *deviations* from the type species of *bancrofti*

Having accepted this view, the next point is whether the atypical forms are *degenerated* forms of *bancrofti*

The first change in degeneration, in my opinion, will be the condition of the granules under staining. The degenerated forms I have observed show a uniform 'peppering' of the body, the granules having lost their true contour. Apart from this change, there is no visible morphological structure which I think would show a serious change under the ordinary methods of examination.

Another point against the view that these are degenerated forms is, that it is unlikely for a particular area in the Balasore district to show a change of this character. The whole epidemiological evidence is against this view.

The question now remains whether they are *independent* forms. In other words the argument is, whether the forms are the offspring of a female of the *bancrofti* species or of another.

In the first instance they will still retain their connection with *bancrofti* and hence they could be styled atypical forms of *bancrofti*. In the second instance they would belong to a different species and would be entitled to be named as such. This point will remain open to doubt so long as the parental forms are not examined.

In addition to this direct method subject to confirmation, there are three points which are in favour of their being classified as new species.

(a) Morphological. The general measurements, the character of the granules, A P and tail should be sufficient grounds to classify them as a separate species of the genus *Filaria*.

(b) Blood picture. Under the heading of relativity of atypical and typical forms in the peripheral blood, I have shown that 63 per cent of cases showed pure atypical forms, the average count per slide being 37. The percentage of cases and the numerical value per slide should prove to be of some significance.

(c) The evidence of *Filaria malayi*. In the 7th session of the F E A T M held at Calcutta in December 1927, Colonel Brug read a paper on '*Filaria malayi* (n sp.) Parasitic in Man in the Malay Archipelago'. Col Brug very kindly gave me a copy of his manuscript along with some specimens of his type species. The average measurements of four specimens were as the following —

C S,  $6 \times 4$ , N, 34, Ex P, 48, A P, 128, L S Z, 156, Diff L S Z — S, 12, Total length, 168

Brug's own measurements in percentage figures as far as I can make out from his diagrams were the following —

N, 19.8—21.8, Ex P, 28—30, A P, 76—80, L S Z, 92—93.8

Both mine and Brug's measurements of *F. malayi* agree in detail.

The measurements of the atypical forms (20 in number) in percentage figures are as the following —

C S, average 6, N, 19.6, Ex P, 28.6, A P, 77.5, L S Z, 93, S 7

These figures agree in the essentials with *malayi*.

The positive rate was appreciably lowered by Kahn and Microkahn. Out of the same 1,000 sera, 425 were positive by Wassermann, 380 by Kahn and 382 by Microkahn.

Table I gives this information at a glance together with an analysis of cases showing relative agreement.

The following conclusions have emerged —

- 1 The Kahn test is not as sensitive as Wassermann.
- 2 For checking the Wassermann the Microkahn is better than the Kahn as it brings out more absolute differences.
- 3 The Kahn and Microkahn tests bring out practically the same number of positive cases. These cases, however, are not identical. Table II makes this point clear.

### III COMPARISON OF RESULTS IN CASES IN WHICH THE CLINICAL CONDITION WAS SPECIALLY INVESTIGATED

In 668 cases there was either a clear history, the patient was seen and the case investigated personally, or a special enquiry form was sent out asking for additional details pertaining to the individual cases. Fifty-six cases showed no agreement. The details are given in the following summary —

Presumption of syphilis	No presumption of syphilis	Treated cases.	History indefinite
13	3	6	34
W R, positive, Kahn, negative, 12	W R, positive, Kahn, negative, 1	W R, positive, Kahn, negative, 2	W R, positive, Kahn, negative, 21
W R, negative, Kahn, positive, 1	W R, negative, Kahn, positive, 2	W R, negative, Kahn, positive, 4	W R, negative, Kahn, positive, 13

In 2,002 cases a similar comparison was made between the Wassermann and Microkahn tests. Two hundred cases showed no agreement. The details are given in the following summary —

Presumption of syphilis	No presumption of syphilis	Treated cases	History indefinite
64	16	20	100
W R, positive, Kahn, negative, 57	W R, positive, Kahn, negative, 4	W R, positive, Kahn, negative, 8	W R, positive, Kahn, negative, 62
W R, negative, Kahn, positive, 5	W R, negative, Kahn, positive, 12	W R, negative, Kahn, positive, 12	W R, negative, Kahn, positive, 36

## EXPLANATION OF PLATE LXXXIII

The Morphological details of *atypical* forms in the human phase

(All microfilaria are sheathed)

- Fig 1 Atypical form of *bancrofti*, attitude inflexible length,  $165 \times 7$  M, granules non-countable, notable areas, I K (colouring and dotting in the white area not shown), A P, L S Z and tail Structures of Ex Z and G<sup>1</sup>Z not visible Scale 1
- „ 1A C S, dotted, showing the nature of the commencement of coarse granules Scale 2
- „ 1B Showing area of A P, L S Z (a coalesced granule), bulbous and dotted tail Scale 2
- „ 2 Showing *bancrofti*, for comparison, attitude, a graceful curve, length  $235 \times 6$  M, granules, 244, L S Z, distinct, tail, like bent finger dotted with one granule, the rest of the structures not shown but are typical of the species Scale 1
- „ 3 Showing what appears to be atypical form, from the same preparation from which Fig 2 is drawn The areas of C S, N, Ex P, I K and A P are shown G<sup>1</sup>Z is seen, part of the dotted tail hidden, for comparison only Length, above  $145 \times 6$  M Scale 1
- „ 4 Atypical form, inflexible attitude, dotted C S, length,  $165 \times 5$  microns, granules, coarse and non-countable, area shown, N, Ex P, I K, A P (distinct structure), L S Z (a coalesced granule), bulbous and dotted tail Scale 1
- Figs 5, 6 and 10 Description like Fig 4 Length,  $162-170 \times 5-6$  M, Scale 1
- Fig 7 Showing C S and commencement of the granules Scale 2
- Figs 8 and 9 Showing portion of extremity from A P to S, the character of L S Z, bulbous tail, as per description in the text Scale 2
- Fig 11 Tail of Fig 10 magnified Scale 2
- „ 12 Showing atypical but apparently transitional form of *bancrofti*, in the same preparation typical *bancrofti* is seen Ex Z, area of I K, G<sup>1</sup>Z, G<sup>2</sup>Z and A P distinct, tail like *bancrofti* but dotted, attitude flexible Length  $162 \times 5$  M Scale 1

2 *Wassermann*

The amount of complement employed in the method described was rather small. There were, however, very few 'one-plus' cases and 'plus-minus' cases in the series. All the 'two-plus' cases would have been positive by methods I, III and IV of the Medical Research Committee (British, now, Council)

3 *Variables in Wassermann and Kahn*

That 'a serum containing a comparatively small number of reacting substances may vary from negative to as high as two plus' and that 'these variations are likely to occur more often in the Wassermann test due to the variables inherent in the hæmolytic system'(13) are well known. The fact, however, that 'certain lots of the beef heart might vary to such an extent that the simple titration method based on dilution with salt solution would not be adequate to ensure uniform sensitiveness of the antigen'(14), in Kahn, is not generally appreciated. Kahn antigen prepared and titrated according to the instructions is not a constant chemical reagent. Its ultimate potency must be determined with the aid of ten known sera and comparison with the standard antigen(15)

4 *Utility of Kahn*

Certain presumptive cases of syphilis missed by Wassermann are certainly detected by Kahn. On this account it is desirable to employ it in conjunction with the Wassermann test. Again, a certain number of cases noted as doubtful by the Wassermann will be found to be definitely positive or negative by the Kahn test. At the Pasteur Institute of Burma, Rangoon, the Microkahn is now done as a routine check in conjunction with the Wassermann. In outlying localities in India and Burma where well equipped laboratories do not exist, the Microkahn might be done alone preparatory to a Wassermann being undertaken, when possible, later.

5 *Will a negative Wassermann and a negative Kahn exclude syphilis?*

Last year, at Kasauli, the writer obtained the following results with three tests —

Wassermann		MICROKAHN			SACHS-GEORGI *		
		Positive	Doubtful	Negative	Positive	Doubtful	Negative
Positive	21	10	3	8	15	4	2
Doubtful	4	1	1	2	0	0	4
Negative	39	1	2	36	4	7	28
TOTAL	64	12	6	46	19	11	34

\* As given by MacKie and McCartney (15)

Two cases were completely negative by Wassermann and Microkahn and fully positive by Sachs-Georgi



	Kahn.	Microkahn.
Absolute agreement	69 6 per cent	66 3 per cent
No agreement	7 "	7 6 "
Relative agreement	23 4 "	26 1 "
	<hr/> 100 "	<hr/> 100 "

## Analysis of cases showing relative agreement

Wassermann, positive, Kahn, doubtful	5 9 per cent	Wassermann, positive, Microkahn, doubtful	6 6 per cent
Wassermann, negative, Kahn, doubtful	4 6 "	Wassermann, negative, Microkahn, doubtful	6 6 "
Wassermann, doubtful, Kahn, positive	3 "	Wassermann, doubtful, Microkahn, negative	3 9 "
Wassermann, doubtful, Kahn, negative	9 9 "	Wassermann, doubtful, Microkahn, negative	9 "
	<hr/> 23 4		<hr/> 26 1 "

TABLE II

Giving the results of Kahn and Microkahn reaction of the same 1,000 sera.

	KHAHN.	MICROKAHN		
		Positive	Doubtful	Negative
Positive +++++, ++++ or ++	380	345	34	1
Doubtful + or ±	141	36	76	29
Negative	479	1	58	420
TOTAL	1,000	382	168	450

Absolute agreement	..	84 1 per cent
No agreement		0 2 "
Relative agreement		15 7 "

## Analysis of cases showing relative agreement

Kahn, positive, Microkahn, doubtful	3 4 per cent
Kahn, negative, Microkahn, doubtful	5 8 "
Kahn, doubtful, Microkahn, positive	3 6 "
Kahn, doubtful, Microkahn, negative	2 9 "
	<hr/> 15 7 "

## REFERENCES

(1) KAHN, R. L. (1925)

(2) *Idem* (1925)

(3) *Idem* (1927)

'Serum Diagnosis of Syphilis by Precipitation'  
Baltimore, Williams and Wilkin Co  
*Ibid*, p 129  
*Jour Amer Med Assoc*, 26th, p 1842



frame work fixed by hinges to the cage so that each can be opened at will for feeding and other purposes. The attendants who have to deal with infected animals are supplied with thick canvas overalls and wicket keeping gloves as a protection against accidental scratches, etc. They are inspected before all operations to see that they are properly dressed for the work (Plate LXXXIV, fig 2)

## *II The Handling of Infected Monkeys*

For the inoculation of infected monkeys, a special box has been designed which practically obviates the actual handling of the animal by the attendants. The box (Plates LXXXIV and LXXXV, figs 1 and 3) is 20 inches by 16 inches by 18 inches in size and is similar to the box for handling squirrels described by me (*I J M R*, Vol XV, No 1, July 1927). Both boxes work on the principle of confining the animal to the smallest space possible by means of a sliding panel after it has entered the box. In the case of the monkey box, the space left when the sliding panel is shoved home is 6 inches by 6 inches.

The part which passes through the slit at the back of the box and which acts as the 'handle' to operate the panel is made the whole height of the box for purposes of strength. The front of the box has two sliding doors, one on each side of the middle line which are locked in either the shut or open position by bolts at the top of the box. Two sets of handles are fixed on each side of the box in convenient places. Projecting from the front on each side are two wings each  $6\frac{1}{2}$  inches wide. These protect the attendants from any possible attack from the monkey while they are lifting the cage.

In addition to the box itself there are two auxiliary pieces of apparatus labelled A and B in Plate LXXXIV, fig 1. These are used to shut off the upper (and dangerous part) of the monkey once he has been caught inside the box. The piece B consists of a flat piece of wood just broad enough to fit under the panel of the box with a handle at one end and a semi-circular notch cut out of the other, large enough to encircle the monkey's body but no more. Two or three of these are made with different sized notches to fit monkeys of different sizes. A small rectangular hole is also cut in this piece in such a position as to be just outside the bars when the end is up against the sliding panel inside the box.

The piece A consists of a piece of wood with a handle at one end and with a small projection at the other to fit into the hole in B when the latter is in position.

The piece B slides along an elevated groove which runs between the two central bars of the box from before backwards. A slides in a groove in the front of the box through the slit which can be seen in Plate LXXXIV, fig 1c, so as to interlock with B.

The whole idea of these two pieces of the apparatus is to shut the monkey's head and arms off into a closed area while leaving his abdomen and legs free for manipulation. The two pieces of wood A and B, the sliding panel and the floor of the box are all lined with zinc for cleaning and purposes of disinfection.

# SOME PATHOLOGICAL ASPECTS OF CHRONIC APPENDICITIS

## Part III.

### THE HISTOLOGICAL DIAGNOSIS OF CHRONIC APPENDICITIS

BY

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THE question has often been asked what is a chronic appendix and how is one to determine if an appendix removed by the surgeon is chronically inflamed. This problem is most important since appendices have been removed and are still being removed in numbers in cases of gastric and duodenal ulcer and in cases of dyspepsia ever since Moynihan drew attention to gastric mimicry in appendix disease. This makes it all the more difficult for the pathologist since no exact diagnostic features have been drawn up and very varying opinions are held as to what a chronic appendix is. In some hospitals many of the appendices removed are reported by pathologists as chronically inflamed and surgeons rest satisfied till the patients subsequently come back with the same symptoms.

To the pathologist the chronically inflamed appendix with marked thickening of the sub-mucosa, atrophy and disappearance of the lymphoid tissue, atrophy of the mucous membrane and even obliteration of the lumen with marked increase of fat and the presence of pigment and even changes in the vessels—an appendix with such well-defined changes—offers no difficulty in diagnosis. But the question has often been raised whether this chronic obliterative fibrosis is the result of previous acute attacks, a view which is held by V. Redwitz and Aschoff, or whether the change is merely an involutary fibrosis. Apart from this question, however, there exists a large number of border line types in which the changes in the submucosa are neither obvious nor sufficiently well marked. These are the types which puzzle the morbid histologist with the result that his report very often is entirely a question of individual opinion.

The histological diagnosis of chronic appendicitis based on the presence of round-celled infiltrations in the submucosa is open to the objection that such cells



With regard to the presence of blood pigment in the submucosa it certainly indicates the presence of blood in the tissue at some time or other. This may mean that there had been a previous hemorrhage and suggests the possibility of previous inflammation. The presence of large numbers of red cells in dilated vessels obviously indicates recent active congestion. With regard to the presence of red blood cells outside the vessels in the submucosa this would indicate recent hemorrhage but for the fact that during section cutting some of the red cells inside the vessels may have been dislodged into the tissues. The table below shows the comparative frequency of blood pigment in cases of chronic appendicitis as compared with the normal.

*Blood pigment in the submucosa of the appendix*

Number of diseased appendices examined	Number in which blood pigment was present in the submucosa	Percentage
175	27	15.6
Number of normal appendices examined	Number in which blood pigment was present in the submucosa	Percentage
235	9	3.8

I have, in a previous paper indicated the tendency of the lymphoid tissue to atrophy and the germ centres to become less defined in chronic appendicitis. This, however, is not of much diagnostic importance but taken together with other features may be of value in deciding in those cases in which well defined features of chronic inflammation are not in evidence.

The presence of fat in the submucous tissue has been generally noticed in cases of chronic appendicitis, but I have found marked increase more commonly in cases of obliterative fibrosis. Fat is usually present in the submucosa near the mesenteric attachment in relation to the gap in the musculature called the *Hiatus muscularis*, through which the submucous tissue becomes continuous with the sub-serous and mesenteric fat. Hence it is rational to assume that fat would be increased in the submucous tissue in conditions where there is increased storage of fat in the normal storage areas and consequently in the mesentery. Out of 175 normal appendices I have examined, fat in the submucosa was found in 93 cases. In chronic appendicitis out of 126 sections examined, fat was found in 73. It is obvious that the percentage of cases in which fat is well marked, remains more or less the same whether the appendix is chronically inflamed or not. I do not, however, refer to cases of obliterative appendicitis in which an increase of fat is a striking feature.

*Endarteritis obliterans* and—patchy intimal thickening—athero-sclerosis—have all been described in chronic appendicitis. In my series of cases, however, well defined changes in the vessels have been found only in cases of chronic obliterative fibrosis. It is true that young arterioles are commonly met with in the submucosa, but they do not usually show any definite evidence of thickening.



# OBSERVATIONS ON THE ATYPICAL VARIETY OF *BANCROFTI* AND ITS SIGNIFICANCE \*

## Part IV.

BY

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[Received for publication, December 11, 1928]

ABOUT two years ago I drew attention to the morphological differences in the embryos of *bancrofti* collected at Muzaffarpur, differences which would form sufficient ground to class the parasites as *distinct variations from the type species* (Korke, 1927)

During the present investigation in Bihar and Orissa, forms of *bancrofti* have been found in the district of Balasore (Orissa), which were not only variations from the type species but possessed a certain degree of correlation with the prevalence of elephantiasis of the extremities as found in that area

These forms are termed as 'atypical' in the present paper to distinguish them from the type species of *bancrofti*, the 'typical'

### MATERIAL AND TECHNIQUE

The material was collected from the agricultural section of the male population of the Balasore district area. The section is usually stationary. The investigation was made in the months of June and July 1928.

Balasore is a sea-coast area on the Bay of Bengal and divided into three parallel tracts of land running from north to south. The eastern tract is a coastal or 'saline area', the middle, the arable area, and the western, a sub-montane area. The district is well watered by the annual rainfall (60 inches), rivers and canals. The mean temperature ranges between 74 to 98°F according to the seasons. Humidity 79 to 89 per cent April-May to August. Paddy is the chief cultivation of the district.

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\* This paper is in continuation of Part III which appeared in the January 1929 number of the *Ind Jour Med Res*

# THE COMPOSITION OF VESICAL CALCULI

*(Preliminary Report)*

BY

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## INTRODUCTION

BY

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[Received for publication, December 17, 1928]

### *Introduction*

IN the course of an investigation of stone-in-the-bladder carried out under my direction during the past few years\* the need for more accurate and extended knowledge of the chemical character of vesical and renal calculi became apparent. A perusal of the literature revealed the unsatisfactory state of this knowledge, while the varying climatic, regional hydrotelluric and dietetic problems involved in the study of urinary lithiasis appeared to have received but scant attention from investigators. Nothing very definite seemed to be known regarding the varying factors which result in the deposition of such different materials as cystin, xanthin, uric acid, urates, ammonium salts, carbonates, oxalates and phosphates, nor of the manner and order of their deposition in different calculi. Little experience was needed to show that, contrary to the dictum of some textbooks, the shape and general appearance of a stone were uncertain criteria on which to rely for an estimate of its composition. These and other matters appeared to render it desirable that the facilities which India affords, not only for the study of urinary calculi as chemical entities but for their investigation with special reference to

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\* *Indian Journal of Medical Research*, Vol XIV, p 895, XV, p 197, XV, p 485 and XV, p 801

In addition to the above diagnostic points, the microfilariæ are sheathed, show a nocturnal periodicity and there appears to be a definite relation between the total number of countable granules and the length in microns both in the human and mosquito phases (Korke, 1928). These are the main features of the type species of *bancrofti* as observed in the areas of Bihar and Orissa.

*Atypical Forms* (see Plate LXXXIII)—The average measurements of 20 forms are given here for the following description. All the parasites are sheathed.

Total length—the average length of the parasites is 178 M, variation, 152—202 M, variation in breadth, 4.5—7.0 M. Thus the parasites are much shorter and slightly thicker than *bancrofti*.

C S—The cephalic space is  $5 \times 3.5$  M, variation,  $4-6 \times 3-5$  M. The length of the space is shorter than *bancrofti* in which the space is more oblong in measurements.

Granules—The granules are coarse, coalescing, non-countable, rhomboidal or spheroidal in shape tending to fill the body cavity, thus differing considerably from *bancrofti*.

N—The nerve ring is an oblique parallel and complete break in the continuity of the granules situated at an average distance of 35 M from the cephalic extremity, variation, 31—45 M or 19.6 per cent. It is anteriorly slightly placed as compared with *bancrofti*.

The most notable anatomical structures after N are Ex P, A P and S, the rest of the structures, i.e., Ex Z, I K and G'Z are difficult to define in all the specimens in a uniform manner so as to be of any value in measurements.

Ex P—The excretory pore is a broad oval break situated at an average distance of 51 microns, variation, 45—66 M, or 28.6 per cent. It is more anteriorly placed as compared with *bancrofti*.

A P—The anal pore is a well defined visible structure enclosing a broad clear oval space. The average distance is 138 microns, variation, 112—169 microns or 77.5 per cent of the total length. More anteriorly placed than in *bancrofti*.

L S Z—The last tail cell is a coalesced granule situated at an average distance of 166 microns, variation, 138—189 M or 93 per cent of the total length. The average distance between L S Z and S is 12.5 M, variation, 10—16 M or 7 per cent of the total length. Thus the tail is longer than *bancrofti*.

S—The tail is the characteristic feature of the atypical forms. Instead of uniformly tapering and bent like a little finger as in *bancrofti*, it is an abruptly drawn out structure, assumes sometimes a bulbous shape towards the middle and is then reflected at an angle or runs almost straight. The bulbous and the end portion is dotted with well defined granules, usually one in the bulbous portion, occasionally 1 to 3 in the end portion.



with 3 per cent potassium nitrate solution, dissolved in a measured amount of N/7 sodium hydroxide solution and the excess back titrated with N/7 nitric acid. From this the *phosphate* was calculated  $P_2O_5$ .

- (2) To a second aliquot part, ammonia, ammonium chloride, ammonium oxalate and acetic acid were added and the mixture allowed to stand 12 hours. The precipitate was filtered off, dried, ignited and weighed giving the amount of *calcium* reckoned as CaO.

To the filtrate sodium phosphate and ammonia were added and any further precipitate filtered off, dried, ignited and weighed as magnesium pyrophosphate, giving the amount of *magnesium* reckoned as MgO.

- (3) To a third part hydrochloric acid and barium chloride were added and any precipitate noted as *sulphate*. In no case was the amount of sulphate considerable.

The *total nitrogen* was determined on a fresh portion of the dry stone by Kjeldahl's method.

The *oxalates* were determined on a third portion of the dry stone, by dissolving in dilute hydrochloric acid (1 in 4), adding ammonia, ammonium chloride, acetic acid and calcium chloride. The precipitate after standing was filtered off, washed, dissolved in dilute sulphuric acid and titrated with permanganate.

The results are given in Table I.

#### The average composition of stones

Taking all the 100 analyses, the average composition of the stones works out to —

Total ash	26.4
Insoluble ash	1.9
$P_2O_5$	7.9
CaO	12.8
MgO	2.3
$SO_4$	trace
	<hr/>
	24.9
$C_2O_3$	11.8
N	19.1

The average of ash not accounted for is 1.5 per cent and is given separately for each stone in column 11 of Table I. In most cases the total of the involatile constituents is within a fraction of a per cent of the total ash, but in a few of the stones there is a considerable difference. This difference is very likely due to

TABLE I

*Showing the distribution of the forms in association with the elephantoid and hydrocele conditions in the Balasore district area*

Area	Total persons examined	Total cases signs, symptoms	Number cases hydrocele	NUMBER CASES ELEPHANTIASIS		CASES SHOWING FORMS <i>bancrofti</i>	
				Upper	Lower	Atypical	Typical
Jalleswar	106	8	1		5	24	
Soro	117	83		2	82	15	
Basudebpur	108	27	9		6	28	3
Balasore town	467	44	15	1	18	47	29
Chandipur	42	2			2	5	6
Bhadrak	610	22	20			2	75
TOTAL	1,450	186	45	3	113	121	113
Percentage		12.8	3		8	8.3	7.8

To support the inference under No (2), I recall the evidence as obtained in the Gaya and Patna district areas (Korke, 1928)

Out of 1,625 cases examined, only 289 or 17.7 per cent showed any signs and symptoms, 155 or 9.5 per cent showed affections of tunica vaginalis, and 13 cedema of upper extremity and 50 of the lower or about 3.9 per cent

The microfilaria found in the peripheral blood belonged to the type species of *bancrofti* and no atypical forms were observed in the above areas

#### *D Relative Numbers of Atypical and Typical Forms in the Peripheral Blood*

Broadly speaking, the peripheral blood was examined in the quantity of 20 cmm taken usually during the hours of 6 and 11 P.M. While examining the preparations, it has been observed that the forms appeared in the blood more often as a representative of one type only

The total cases selected for this observation were 74. The average count of the parasite per slide from each area in the Balasore district is as the following (Table II)

The inferences to be drawn from the above table are the following —

(1) That out of 74 persons, 63 per cent of cases showed pure atypical forms, the average count per slide being 37 parasites

(2) That 27 per cent of cases showed pure typical forms, the average count per slide being 20

*" The Composition of Vesical Calculi.*

TABLE I—contd

1	2	3	4	5	6	7	8	9	10	11
Stone number	Moisture as percentage of original stone	AS PERCENTAGES OF THE DRY STONE								
		Insoluble		Total nitrogen	P <sub>2</sub> O <sub>5</sub>	Ca O	Mg O	SO <sub>4</sub>	C <sub>2</sub> O <sub>3</sub>	Ash not accounted for
		Total	Insoluble							
57	84	171	Trace	253	63	75	29	Nil	66	04
58	13	37	Nil	301	Trace	34	Trace	Nil	39	03
59	13	41	Nil	283	Trace	38	Trace	Nil	62	03
60	175	654	15	29	260	295	72	Nil	151	12
83	50	390	Nil	40	49	320	10	02	378	11
84	20	87	Nil	254	Trace	81	01	Nil	98	05
85	22	215	Nil	152	10	197	01	Trace	238	09
86	124	526	Nil	17	148	332	37	Nil	279	04
100	16	58	Nil	270	Trace	54	Nil	Present	69	71
102	38	521	205	36	Trace	245	Nil	Nil	306	09
103	15	42	Trace	294	Trace	33	Nil	Trace	42	04
104	110	635	398	39	64	108	Trace	Nil	118	65
119	17	107	Nil	250	21	83	Trace	Trace	88	03
120	28	221	Nil	218	75	130	Trace	Trace	80	16
121	56	468	Trace	21	98	330	14	Trace	361	26
123	119	212	Nil	261	94	51	46	Trace	32	21
131	19	43	Nil	305	Trace	29	Trace	Nil	35	14
137	36	134	Nil	205	Nil	127	Trace	Nil	165	07

4 cases from Soro and Balasore area showed elephantiasis in which only atypical forms were observed

The one inference is, that the relation between the typical form and affections of tunica vaginalis is of frequent occurrence

The evidence regarding the relation between atypical forms and elephantiasis is slender on the finding of the parasite in the peripheral blood alone, but becomes stronger when one observes the large number of elephantiasis cases in the Soro area where only atypical forms so far have been observed (Table I)

#### F Distribution of the Atypical Forms with the Species of Mosquitoes

It has been observed that the atypical forms are found largely in numbers in the Jalleswar and Soro areas. The species of mosquitoes investigated in the Bihar and Orissa area have already been recorded in Part III (Korke, 1929). I would therefore only mention here the species that are found in the Soro and Jalleswar areas

Soro	<i>Tæmorhynchus (Mansomoides) annuliferus</i>
	<i>T (Mansomoides) uniformis</i>
	<i>C vishnu</i>
Jalleswar	<i>T (Mansomoides) uniformis</i>
	<i>Lutzia fuscana</i>
	<i>C fatigans</i>
	<i>C vishnu</i>
	<i>C bitæmorhynchus</i>
	<i>C epidesmus</i>
	<i>C whitmorei</i>

#### DISCUSSION OF RESULTS

*Atypical Forms*—The discussion resolves itself into the following points —

- (1) Are they *deviations* from the type species?
- (2) Are they *degenerated* forms of the type species?
- (3) Are they *independent* forms?

Experience teaches one that in a nematode larva the morphological characters which should be subjected to a close scrutiny are the mouth parts, alimentary canal, genital primordia, anus, the shape and character of the tail, and the condition of sheath

A nematode larva sanguineous in habit like *bancrofti* can only present four of the above mentioned characters for a ready observation. Out of these four characters genital cells in a mass of granules is a structure, which may easily waylay an observer especially under the routine methods of staining and examination. But A P sheath (if present) and the character of a tail will not escape notice even under casual observation. The character of the granules, periodicity and anatomical measurements are additional points to confirm a species

TABLE I—*contd.*

1	2	3	4	9	5	6	7	8	10	11
Stone number	Moisture as percentage of original stone	AS PERCENTAGES OF THE DRY STONE.								
		ASH.		Total nitrogen	P <sub>2</sub> O <sub>5</sub>	C O	Mg O	SO <sub>4</sub>	C.O. <sub>2</sub>	Ash not accounted for
		Total	Insoluble							
6	12	27	Trace	308	Trace	22	01		Trace	04
8	144	635	348	41	102	123	59		136	03
10	228	538	Nil	126	278	203	51		15	06
13	97	220	Trace	255	60	81	39		58	40
28	14	02	Nil	329	Trace	02	Nil		00	00
29	36	37	Nil	313	16	07	07		00	07
35	15	28	Nil	306	Trace	26	Trace		31	02
39	16	23	Nil	318	Trace	21	Trace		25	02
48	13	53	Nil	286	Nil	52	Trace		59	01
49	52	510	210	43	38	190	Trace		236	72
50	80	434	Trace	10	58	350	06		405	20
52	23	69	Trace	280	Trace	61	Trace		68	08
62	28	433	Trace	07	Trace	371	Trace		462	62
64	21	143	Trace	203	Trace	140	01		178	02
65	77	850	Trace	68	364	445	13		41	28

On the epidemiological evidence Brug has found a wide prevalence of *malayi* in the archipelago in association with elephantiasis

I have suggested before that, although the evidence on the latter point in the Balasore district is slender in the case of atypical forms, it tends more towards that direction

An important argument against the view that the atypical forms are independent forms is the question of adaptation of the type species of *bancrofti* to the culicine mosquitoes prevalent in the area of Balasore. I have no definite evidence on this point, but Manson-Bahr's work in Fiji (Bahr, 1912) precisely emphasizes the difficulty which one encounters in classifying the microfilariae when certain characters, may be of morphology or of periodicity, are modified by the local circumstances and the habits of the carrier

Till this point is definitely settled, I consider myself justified in retaining the name of the parasites as *atypical* forms of the type species of *bancrofti*

### CONCLUSIONS

1 That forms resembling the embryos of *bancrofti* have been observed in the Balasore district area (Orissa) possessing morphological characters which were *distinct variations from the type species* and are termed *atypical* forms

2 Elephantiasis of the lower extremity appeared to be more prevalent in the areas where the *atypical* forms were found in numbers

3 Affections of tunica vaginalis (hydrocele) appeared to be more prevalent in the areas where typical forms of *bancrofti* were found in numbers

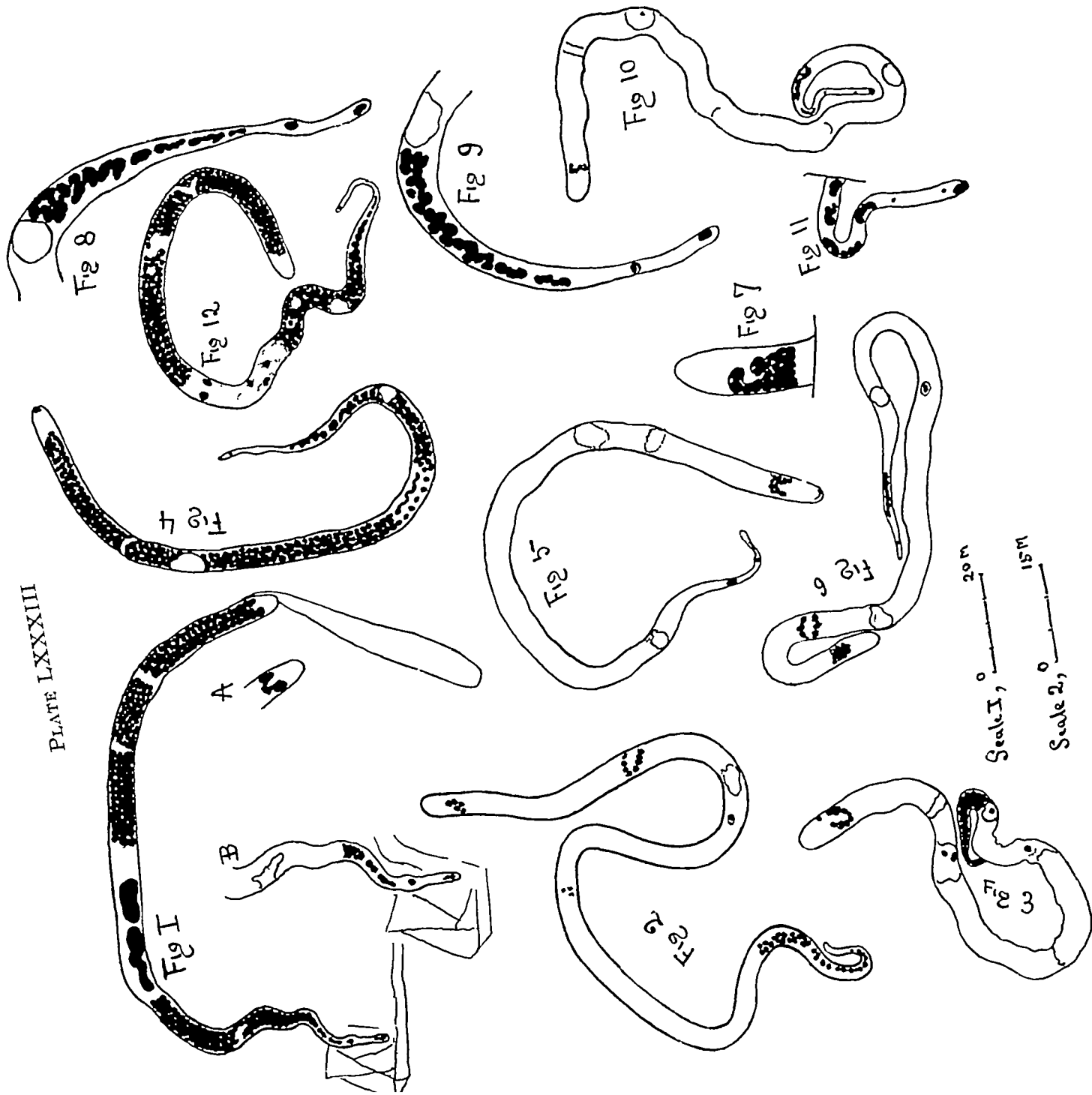
4 The characters of the parasites very nearly resemble *Filaria malayi*, (n sp., Brug, 1927), the reasons for classifying them for the present as *atypical* forms of *bancrofti* have been given fully in the body of the paper

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- |                                  |   |
|----------------------------------|---|
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| BENGAL DISTRICT GAZETTEER (1907) | Balasore.   |
| BRUG (1927)                      | <i>Filaria malayi</i> n sp Mss Copy                     |
| KORKE (1927)                     | <i>Ind Jour Med Res</i> , Vol XIV, No 3, January, p 717 |
| <i>Idem</i> (1928)               | <i>Ibid</i> , Vol XVI, No 1, July, p 187                |
| <i>Idem</i> (1929)               | <i>Ibid</i> , Vol XVI, No 3, January, Part III          |

TABLE I—*concl'd*

1	2	3	4	9	5	6	7	8	10	11
Stone number	Moisture as percentage of original stone	AS PERCENTAGES OF THE DRY STONE								
		Ash		Total nitrogen	P <sub>2</sub> O <sub>5</sub>	Ca O	Mg O	SO <sub>4</sub>	C <sub>2</sub> O <sub>3</sub>	Ash not accounted for
		Total	Insoluble							
142	50	55.1	33.2	2.2	Trace	16.8	0.6		21.8	4.5
144	76	44.1	Nil	9.7	11.1	27.9	2.5		20.6	2.6
145	20	9.8	Nil	25.6	Trace	7.2	Trace		12.5	2.6
172	49	37.6	Nil	2.6	2.5	32.5	Trace	0.04	40.7	2.6
173	160	39.8	Nil	13.0	16.9	15.1	5.7	Nil	8.6	2.1
176	42.9	78.7	Nil	13.5	48.8	Nil	28.6	Nil	0.0	1.3
177	15.5	21.6	Trace	25.8	10.9	1.7	5.9	Trace	0.0	..





A large number of different methods were tried for the direct estimation of uric acid in the stones, but none of them was satisfactory. These were briefly —

(i) Lithium, sodium and potassium carbonates were tried as solvents for uric acid in stones and from a series of experiments lithium carbonate was found to be the best and 0.1 per cent solution was a satisfactory strength.

(ii) It was found that for a colorimetric estimation of uric acid with Folin and Wu's reagent it was necessary to have the concentration of uric acid between 0.5 and 2 mg per 100 c.c.

(iii) Thirty-one of the stones finely powdered, were extracted with 0.1 per cent lithium carbonate solution for two hours in the cold, and the uric acid in an aliquot part of the clear supernatant liquid estimated by Folin and Wu's method. At first this method seemed satisfactory, and this was why it was tried so extensively, but afterwards a comparison of the results with the figures got by multiplying the total nitrogen by three showed that the results were quite unreliable. The figures got from the total nitrogen is reliable as a maximum figure, and the figures from this method varied from 50 per cent above to 50 per cent below this figure. The mean of the thirty-one estimations corresponded very well with the mean of the total nitrogen figures (1.081), an additional indication that most of the nitrogen is in the form of uric acid.

(iv) The arseno-phospho-tungstic acid method of Benedict and Franke was also tried on a lithium carbonate extract, but the results, as compared with (iii) and with the total nitrogen, were entirely irregular.

Failing to estimate the uric acid directly, another method of dividing up the total nitrogen was tried.

(1) In such stones as from qualitative tests it was suspected that some of the nitrogen was in the form of ammonium salt or amide, the amount of this was estimated by boiling a weighed amount of the powdered stone with alkali in a Kjeldahl's apparatus and back titrating the ammonia evolved. The results are given in Table II.

(2) In those of the stones which failed to give the murexide test (most of the stones with low nitrogen) a weighed quantity of the powdered stone was digested for an hour with 0.1 per cent lithium carbonate solution and the nitrogen in the insoluble residue estimated by Kjeldahl's method. It was thought that proteins would be insoluble in boiling lithium carbonate solution, while uric acid would be dissolved out. There are very likely other nitrogenous compounds in stones besides proteins and uric acid and these might go into solution or remain in the residue so that the method cannot be considered very satisfactory but it gives an indication that in those of the stones examined a small amount of the nitrogen is in the form of protein. The results are also shown in Table II.

Many of the stones were tested for creatinine by Jaffe's test but in no case was any detected.

# A NEW TECHNIQUE FOR HANDLING INFECTED MONKEYS

BY

LIEUT-COL J CUNNINGHAM, C I E , B A , M D , I M S ,

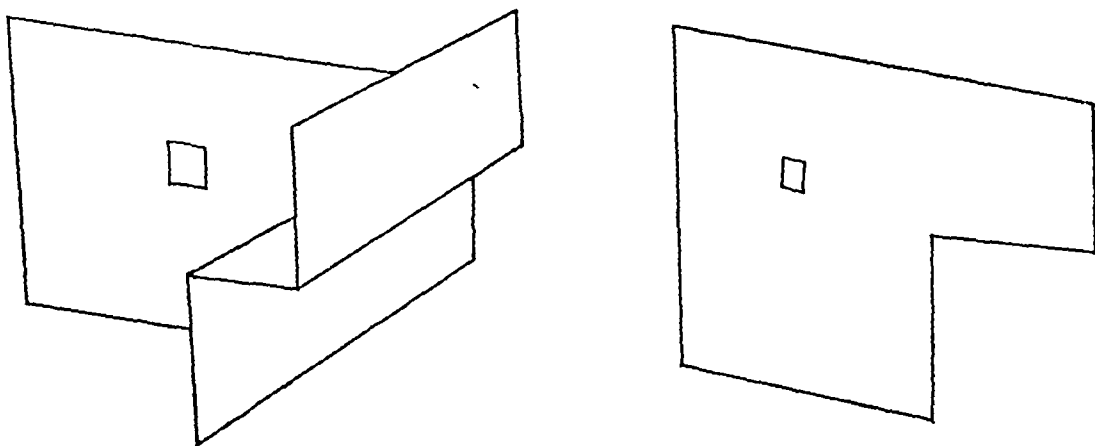
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[Received for publication, December 17, 1928 ]

THE anti-rabic experiments at present being carried on at the Pasteur Institute involve two processes the infection of the monkey and its inoculation after it has been infected In each process the monkey has to be caught No particular danger attaches to the first process as the monkey is not yet infected The inoculation of infected monkeys is, however, attended with considerable risk as many of the animals are in the incubation period of the disease while being inoculated and are thus infective It is for the latter process that a special box has been introduced As I have received several requests for a description of the technique employed for this purpose, the following short note is being published

## *I Monkey Cages*

Infected monkeys are kept in special cages These consist of a wooden frame work with iron bars inserted on all sides including the floor The front has a sliding door The inner corners of the frame work are lined with zinc to prevent the wood from being eaten away In addition these are surrounded on



all sides (except the bottom) with strong wire gauze which prevents any possibility of outside contact The gauze on the front and top is attached to a special

TABLE II—*concl'd*

Stone number	Nitrogen as per cent of dry stone in the form of	
	NH <sub>4</sub>	Protein.
39	2 5	
64		0 5
65	0 7	
67		1 0
74		0 4
79		0 4
90		1 1
91	4 3	
95		0 2
108		0 6
109		0 8
113		1 0
114		0 6
115		0 8
117	3 7	.
128	5 1	2 2
144		0 2
159		0 5
161	4 8	1 0
162		1 0
167		0 6
169		2 5
180		0 2
		Mean 0.92=5.8 per cent protein

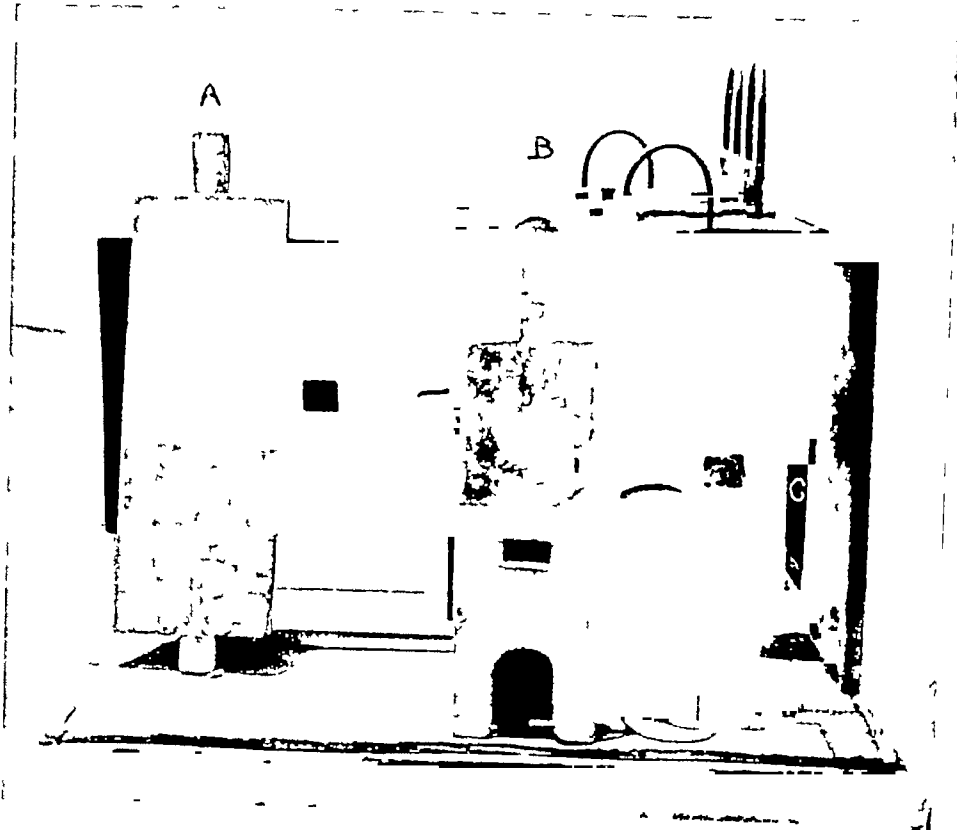


Fig 1

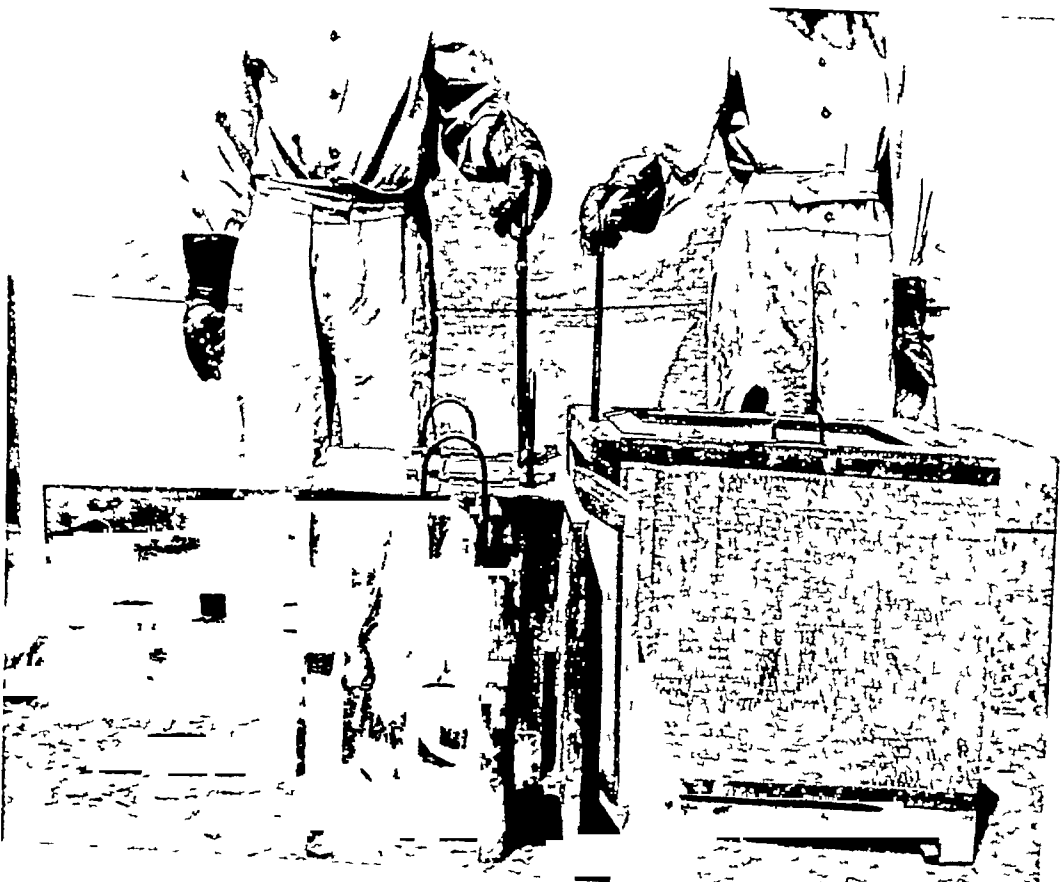


Fig 2

*The classification of the stones*

In view of the above conclusions the stones might well be classified into urate, non-urate and mixed, but as the more usual classification is into urate, oxalate and phosphate stones and combinations of these, this has been adopted. The coarser classification can be easily got from it by a few small additions.

In classifying the stones we have neglected amounts of phosphates and oxalates below 1 per cent and been guided by the murexide test in deciding if stones with only a little nitrogen should be classed as containing urates or not.

The results of this classification, divided into the provinces or states from which the stones came, are shown in Table IV.

TABLE IV

*Showing the composition of stones according to the Provinces or States from which they came*

Provinces	Urate	Phosphate	Oxalate	Urate and phosphate	Urate and oxalate	Phosphate and oxalate	Urate, phosphate and oxalate	Totals
N-W F P	2		1	3	8	0	3	17
Punjab	4			5	6		4	19
United Provinces	1			1	11	2	4	19
Central Provinces			1		1	2		4
Bengal				1		2	1	4
Assam		1			7	1	6	15
Hyderabad	1					1		2
Madras		1	1			1	1	4
Bombay				1	4	3	3	11
Bihar and Orissa	1						1	2
Nepal							2	2
Ajmer-Merwara					1			1
TOTALS	9	2	3	11	38	12	25	100

## SUMMARY

1 A hundred vesical stones from all over India have been analysed.

2 The stones are composed for the most part of uric acid or urates and phosphates and oxalates of calcium and magnesium. In many stones and probably in all, some protein is present.

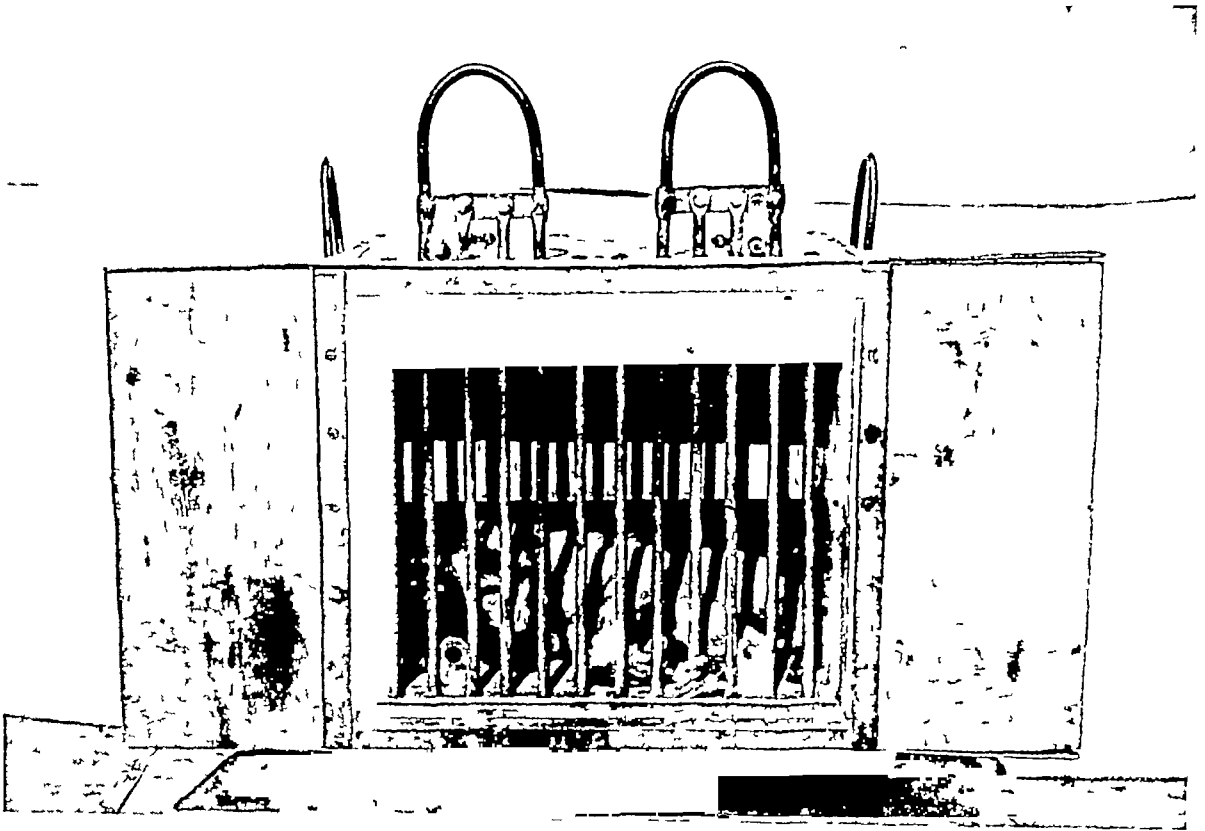


Fig 3

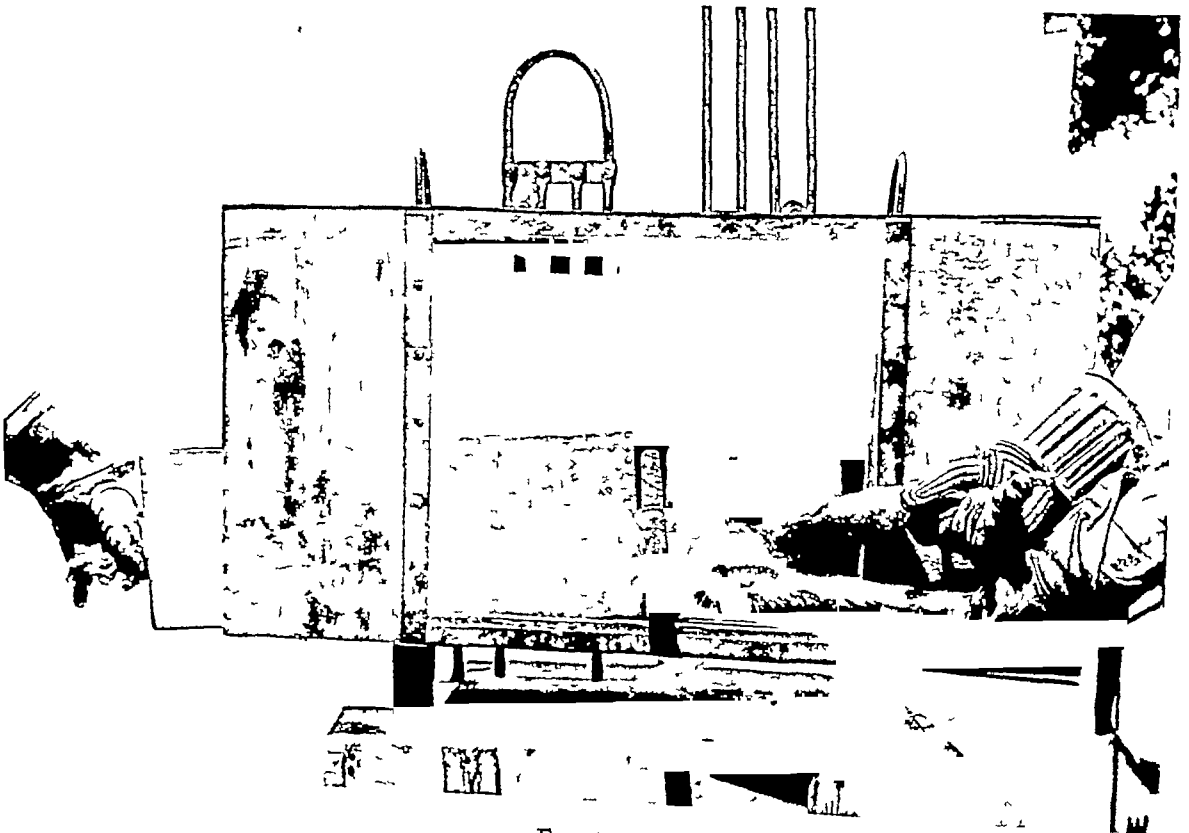


Fig 4

# A REVISION OF THE CULICINE MOSQUITOES OF INDIA

## Part XXV.

### THE GENERA *MUCIDUS*, *MIMOMYIA*, *FICALBIA*, *RACHIONOTOMYIA*, AND *HODGESIA*

BY

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[Received for publication, December 22, 1928]

#### Genus *MUCIDUS* Theo

Theobald, 1901, *Mon Cul*, Vol I, p 268

The two Indian species belonging to this genus may be easily distinguished from other mosquitoes by the presence of outstanding yellow, white, and brown scales on the body and legs, giving them a mouldy and mottled appearance, and also by their comparatively large size. Average specimens are larger than *Lutzia fuscana* (Wied) ('*Culex concolor*'). The wings are mottled with yellow, brown, and creamy scales, the membrane shows some darkening in the region of the cross-veins, and the cross-veins s, r-m, and m-cu, are in a straight line, or m-cu may be slightly nearer the tip of the wing than r-m. There are a large number of pro-epimeral bristles, usually about 20. This character distinguishes *Mucidus* from the closely allied genus *Pardomyia* which, as far as is known at present, has no representative in India. Some of the white scales on the mesonotum are remarkably long and twisted, resembling strands of cotton-wool. The external genitalia of both sexes resemble in structure those of species belonging to the subgenus *Ochlerotatus* of *Aedes*.

The larvæ are predaceous and are usually found in large pools in association with those of various species of *Culex*, *Aedes* (*Aedes*), and *Aedes* (*Aedimorphus*), which live in such situations.

Plate LXXXIV, fig 2, shows the box in position against a case, with the sliding panel withdrawn to the fullest extent and *one* of the doors of the box opposite the iron door of the monkey's cage opened. The box and the cage are kept closely applied to each other by means of catches. The iron doors of the box and monkey cage are now opened and, as a rule, the monkey goes in without any trouble. If he refuses, the top gauge door of his cage is opened and he is gradually forced towards the door by cutting down the space in his cage by means of a board passed between the bars. This is very seldom necessary. Once inside the box the door is closed, bolted, and the box is placed on a table. One attendant gently shoves up the sliding panel and the monkey feeling the pressure generally lies down when the panel is shoved home and locked by passing a bar of wood through a hole in the panel at the back of the box (Plate LXXXIV, fig 1). Very rarely the monkey refuses to lie down. The box can then be turned on its side so that the axis of the confined space takes up the position demanded by the monkey. In practice we have found, however, that the monkey learns to lie down by himself. The piece of wood B is now inserted and shoved home to the sliding panel. A little manipulation may be required here to get the monkey's arms up, but here again this is rarely troublesome. The slab A is then inserted through the slot C and interlocked with B (Plate LXXXV, fig 4). Both of these are now held by one attendant while the other opens the door opposite the lower part of the monkey, bolts it open and withdraws the legs and lower part of the body.

In practice as soon as the legs are drawn upon, the monkey catches hold of the bars with his hands and pulls against the attendant so that his lower part can be freely withdrawn from the box. There is no danger of his being pulled out of the box altogether even if he lets go as long as the attendant keeps the piece B in position as the notch is too small to allow the shoulders and arms of the monkey to pass through. The lower part of the chest, the abdomen and the lower limbs are thus made available for inoculation or any other manipulation required such as the withdrawal of blood from the saphenous vein, spleen or liver (Plate LXXXV, fig 4). Once the operation is concluded the lower limbs are returned to the box, the door shut down, the pieces A and B removed and the monkey returned to his cage in the same way as he was removed from it. Our average time for giving a monkey an inoculation from the time the box is put against his cage until he is returned to it again is 4 minutes. Troublesome animals of course take longer but our longest time with a fighting animal has been 10 minutes. Modifications can easily be introduced according to the type of manipulation required. Thus the notch in piece B could be made smaller so as to fit the monkey's neck and in this way the whole animal would be available if required. Again—one type of box we made had sliding doors at each side behind the wings of the cage, through which the monkey could be withdrawn after the sliding panel had been passed home so that he could not turn.

## REFERENCE

CUNNINGHAM, J (1927)

*Ind Jour Med Res*, Vol XV, No 1, July



There are two males in the Malaria Survey of India collection, Kasauli, probably collected in 1911 in Assam (*Christophers*) but the exact data were not attached to the specimens. It has been recorded from Ceylon (*Edwards, Ind Jour Med Res*, X, 462)

### Genus MIMOMYIA Theo

*Mimomyia*, Theobald, 1903, *Mon Cul*, Vol III, p 304

*Ludlowia*, Theobald, 1907, 1 c, Vol IV, p 193

*Radioculex*, Theobald, 1908, *Rec Ind Mus*, Vol II, p 295

*Conopomyia*, Leicester, 1908, *Cul Malaya*, p 113

The characteristics distinguishing this genus are as follows —fork-cells of wing short, especially the anterior which is only about half the length of the stem, otherwise the venation is normal (i.e., the commencement of vein 2 is nearer the root of the wing than the fork of vein 5, the latter being nearer the root of the wing than the tip of vein 6), microtrichia of normal size, pulvilli absent, post-spiracular area without bristles or scales, no spiracular bristles, several strong pro-epimeral bristles, no lower mesepimeral bristles but about 12 on the upper part of the sclerite, about 12 sterno-pleural bristles. Tarsal claws of the female simple, those on the fore and mid legs of the male toothed, one with two teeth, and one with one, claws of the hind legs simple (only males of *M chamberlaini* have been available for examination). Mesonotal and scutellar scales narrow but the head mainly covered with flat scales. Palpi of the male clubbed at the extremity and as long as, or slightly longer than, the proboscis, those of the female about one-quarter the length of the proboscis.

The male genitalia are of simple structure, ninth tergite with a median lobe bearing a group of three or four long stout bristles on each side, side-piece in a dorsal or ventral view twice or three times as long as the width near the base, covered externally with numerous hairs and with a few scales on the outer sides, a lobe on the inner surface carrying several very strong bristles, phallosome feebly chitinized and without lateral plates or teeth, chitinizations of the anal segment with several strong curved teeth at the crown and a few minute hairs.

Only two species are known to occur in India, *M minima* (Ludl) and *M chamberlaini* Ludl. The former is a small dark species whilst the latter is larger, with yellow scales along the sides of the mesonotum, and with pale rings on the tarsi.

### *Mimomyia minima* (Ludl)

*Ludlowia minima*, Ludlow, 1907, *Can Ent*, Vol XXXIX, p 413

*Conopomyia hybrida*, Leicester, 1908, *Cul Malaya*, p 115

No 1 undetermined species, Leicester, 1908, 1 c, p 260

*Ficalbia ludlowi*, Brunetti, 1920, *Rec Ind Mus*, Vol XVII, p 173,

nom nov for *Ludlowia minima*

climate, racial, dietetic and other conditions of life, should be made use of. Stone-in-the-bladder is very prevalent throughout the Indian Peninsula, being much more so in some localities than in others and amongst certain races and castes than amongst others. Year by year hundreds of calculi are removed at operation, while the rigidity with which certain habits of life and diet are adhered to by the different races comprising the population of India, affords material for comparison which does not exist to the same extent in other countries. Studies made in these laboratories of the relative values of the national diets of India appeared likely to facilitate an investigation of stone from the dietetic point of view. Accordingly, I undertook in 1927 the collection of urinary calculi, addressing, to this end, a circular letter and questionnaire to 51 surgeons practising in different parts of India. I take this opportunity of thanking them for their unanimous and generous response to my appeal. Through their kindness 226 vesical and renal calculi have reached us up to date, together with a complete social, dietetic and medical history of each case.

Learning that urinary lithiasis was a comparatively frequent condition in cattle in certain parts of India, a similar appeal was made, on our behalf, by Mr Frank Ware, Veterinary Advisor to the Government of Madras, to Veterinary Surgeons in the South of India. To him and to them we are greatly indebted. Sixteen urethral calculi from cattle have reached us to date, and it is hoped that a comparison of their composition with that of human calculi may yield results of interest.

The chemical analysis of this material has been undertaken by Major Clive Newcomb and the assistants, Mr B N Banerji, M Sc, and Mr S Ranganathan, B A, in the Chemical Department of these laboratories. Major Newcomb submits a preliminary report on the analysis of 100 human stones in the paper which follows. Further chemical reports will appear at a later date, and from time to time papers will be published dealing with our study of the material we have collected.

#### *Method of analysis*

The stone, if large and whole, was sawn through the middle and half of it, or a slice taking in all the different layers, powdered, with small or crushed stones the whole was powdered. Some of the powder was dried to a constant weight at  $110^{\circ}\text{C}$  and the loss of weight reckoned as *moisture*. The dry powder was then used for subsequent determinations.

Some of the dry powder was ashed at a red heat and the *ash* weighed. The ash was then moistened with water and extracted with dilute hydrochloric acid. If not entirely soluble, the residue was again heated and the extraction repeated. Any residue still insoluble was filtered off, weighed and reckoned as '*insoluble ash*'. Aliquot parts of this acid solution of the ash were taken —

- (1) To one part, nitric acid and ammonium molybdate mixture were added and the precipitate of phosphate—if any—allowed to settle for 12 hours. The precipitate was then filtered off, washed free from acid,

The following records of distribution have been collected chiefly by examination of actual specimens —

Punjab — Amritsar (*Christophers*)

Bihar and Orissa — Pusa, 12 ii 1916 and 30 vi 1927 (*Shaffi*), Katihar, Purneah district, 4—5 x 1908 (*C Parva*), Rambha, Chilka Lake, xi 1922 (*Barraud*)

Bengal — Calcutta, November, December, and July, common amongst brush-wood in the cold weather (*Annandale*), Berhampur, Murshidabad district, 1 i 1908 (*R E Lloyd*), Dacca, xii 1911 (*S K S*), Santragachi, Howrah, 4 viii 1926 (*R Senior White*)

Madras — Ennur (*Major Rose*), Madras (*Patton*), South Kanara (Central Malaria Bureau records)

Nilgiri Hills — Coonoor (C M B records)

Travancore — Yakkam, coastal region, 5 xi 1908 (*Annandale*)

Burma — Rangoon, 25 ii 1908 (*Annandale*)

Ceylon — Colombo (*James*)

#### var *intermedia*

Assam — Gauhati and Jorhat, iii 1923 (*Shaffi*), Nongpoh, vii 1922 (*Barraud*), Golaghat, i and xi 1925 (*Barraud*)

*M chamberlaini* was described from one male from Bayamban, Pangasinan, Luzon, Philippine Islands, 15 v 1904 (*W P Chamberlain*) The type is presumably in the Army Medical Museum, Washington The type male and female of *R clavipalpus* are in the Indian Museum both from Calcutta, 11 xii 1907 and 17 xi 1907 (*Mus collr*)

#### Genus FICALBIA Theo

*Ficalbia*, Theobald, 1903, *Mon Cul*, Vol III, p 296

*Etiroleptomyia*, Theobald, 1904, *First Rept Wellc Lab*, p 74

*Ingrana*, Edwards, 1912, *Bull Ent Res*, Vol III, p 43

The mosquitoes belonging to this genus are of very small size and none of the three species found in India appear to be common or of much importance

The following combination of characteristics serve to distinguish the genus from others — wing scales large, some of them being asymmetrical, as in the mosquitoes included in the subgenus *Mansonioides* of *Taeniorhynchus*, anterior fork-cell rather long, post-spiracular area without bristles or scales, no spiracular bristles, a few strong pro-epimeral bristles, pulvilli absent, proboscis of the male considerably enlarged towards the tip (Plate LXXXVI, fig 4), that of the female only slightly enlarged In the females I have been able to examine (*F minima*) there is only one chitimized spermatheca, as in *Anopheles* females The structure of the male genitalia in this genus and in *Mimomyia*, described above, is very similar In the male of *F minima* the median lobe of the ninth tergite is flat-topped and carries about 12 strong bristles

TABLE I  
Showing the results of analysis

1	2	3	4	9	5	6	7	8	10	11
Stone number	Moisture as percentage of original stone	AS PERCENTAGES OF THE DRY STONE								
		ASH		Total nitrogen.	P <sub>2</sub> O <sub>5</sub>	Ca O	Mg O	SO <sub>4</sub>	C <sub>2</sub> O <sub>3</sub>	Ash not accounted for
		Total	Insoluble							
2	19.0	62.5	0.8	9.5	37.0	23.6	Trace		13.3	1.1
3	1.9	10.1	Nil	25.4	Trace	8.6	Nil		10.9	1.5
4	17.0	38.6	0.4	11.5	17.1	16.7	Trace		19.9	4.3
5	4.5	55.1	19.4	5.7	11.5	19.2	Nil		20.7	5.0
17	1.3	6.4	Nil	28.8	1.0	4.6	Trace		4.5	0.8
18	1.0	0.7	Nil	32.3	Nil	0.8	Nil		0.0	-0.1
19	2.6	9.8	Nil	24.8	1.0	5.6	Nil		6.3	3.2
20	9.3	37.2	Trace	14.2	11.0	20.4	Trace		16.7	5.8
21	18.9	76.4	Nil	2.2	32.8	30.6	8.6	Trace	3.6	4.4
22	1.4	4.5	Nil	30.1	0.5	3.6	Trace	Trace	3.8	0.4
25	1.5	2.8	Nil	30.3	Nil	2.5	Trace	Nil	3.0	0.3
26	11.2	45.8	Nil	8.6	13.8	25.7	3.6	Trace	19.8	2.7
41	5.9	1.2	Nil	32.1	Nil	1.0	Nil	Nil	1.2	0.2
44	1.8	3.6	Nil	29.7	Trace	3.2	Nil	Nil	3.9	0.4
46	1.3	1.1	Nil	32.6	Trace	0.3	Nil	Nil	0.0	0.8
47	3.8	2.2	Nil	30.5	Trace	2.0	Trace	Nil	3.2	0.2

There are a number of specimens in the Kasauli collection from Nalbari, Assam, caught in jungle, ix 1928 (*Sobha Ram* collr) Other available records are —Calcutta, 1 female at light, Indian Museum Buildings, viii 1911 (*Graveley*), Calcutta (*M O T Iyengar*), Calcutta, 1 male, 2 females, xi 1910 (*H N Sharma*), Puri, Orissa Coast, 1 female 24—28 i 1911 (*Annandale* and *Graveley*), Dacca, 1 female, xii 1911 (*S K S*)

Two co-type males of *U minima* are in the British Museum, from Quilon, Travancore, 7 iii 1900 (*James*) Type male and female of *M minuta* are in the Indian Museum, the former from Sylhet, Assam (*Major Hall*), the latter from Calcutta, 30 vii or 4 vii 1907 (*Annandale*)

### Genus RACHIONOTOMYIA (Theo) Edw

Edwards, 1921, *Bull Ent Res*, Vol XII, p 283

Theobald, 1904, *Jour Bomb Nat Hist Soc*, Vol XVI, p 248, and *Mon Cul*, Vol IV, p 518

This genus was erected by Theobald on a misconception and has been redefined by Edwards The generic characters may be summarized as follows — Proboscis very long and slender throughout, longer than the long front femora, and as long as the whole body Palpi quite short in both sexes, not more than one-sixth the length of the proboscis Prothoracic lobes rather large but well separated One pro-epimeral bristle (even this may be absent in some species), from 3 to 6 spiracular bristles, no post-spiracular bristles, no row of bristles along the upper part of the sterno-pleura, no lower mesepimeral bristles Post-notum bare with a pair of slight furrows dividing it into three parts Hind tibiae shorter than the others Tarsal claws of the female simple, claws of the fore leg of the male unequal (in three of the species included in this paper the larger claw has a blunt tooth at about the middle, but in *R powelli* var *n indica* it is simple) Fork-cells moderately long, the tip of vein 6 nearer the apex of the wing than the commencement of vein 2 Parts of the male genitalia are illustrated on the accompanying plate The ninth tergite curves away from the other parts of the hypopygium and is produced into a pair of well-developed submedian lobes bearing strong bristles, flattened spines, or leaflets (Plate LXXXVI, figs 5, 8, 9 and 10) These lobes appear to form a pair of 'inferior claspers' and are homologous with the structures so named in the male genitalia of *Phlebotomus* The side-pieces are comparatively small and there is a hairy lobe on the inner side of each, the clasper arises from the apex of the side-piece, is long, and has a small stout terminal appendage There are several strong teeth at the crown of the chitinizations of the anal segment and a few minute hairs, as is the case in a number of other genera The phallosome is in the form of a feebly chitinized incomplete tube with sometimes a few minute teeth at the apex

The known Indian species are all small mosquitoes, and apparently of little importance, as they are usually only found in heavy forest and jungle where the

138	233	332	29	221	156	14	105	Nil	00	28
140	14	108	Nil	233	Trace	103	01	Nil	124	04
149	105	441	Nil	63	110	280	Trace	Nil	277	51
152	54	410	Trace	29	44	332	Trace	Trace	398	34
163	27	278	Nil	128	39	224	Nil	Nil	246	15
164	17	65	Nil	269	Trace	60	Nil	Nil	74	05
156	200	412	Nil	173	206	107	85		Trace	14
157	357	778	Nil	32	440	133	204		Trace	01
158	10	04	Nil	320	Nil	03	Nil		00	01
159	234	572	Nil	112	297	174	81		00	20
161	40	124	Nil	267	Trace	79	15		107	30
162	18	54	Nil	286	Trace	48	Trace		48	06
165	13	04	Nil	318	Nil	02	Nil		00	02
166	15	63	Nil	270	Trace	58	Trace		72	05
167	350	655	Nil	78	370	71	211		Trace	03
169	37	316	Nil	75	Trace	286	Trace		350	30
170	14	31	Nil	298	Trace	30	Trace		39	01
171	208	408	Nil	180	207	93	99		00	09
175	16	47	Nil	295	Trace	38	Trace		43	05
178	30	161	Nil	221	Trace	139	05		152	17
179	15	60	Nil	287	Nil	58	Nil		67	02
180	17	97	Nil	243	Nil	93	Nil		119	04

Eastern Himalayas—Sureit and Mungpoo, x 1922 (*Barraud*)

North Bengal—Sukna, ix 1922 (*Barraud*)

Assam—Shillong vi and viii 1922 (*Barraud*), Haflong, viii 1922 (*Barraud*), Golaghat, v 1925 (*Barraud*), Khumtai (*Christophers*)

Burma—Rangoon, i 1920 (*Christophers*)

Andamans—vii 1926 (*Sobha Ram collr*)

Ceylon—Colombo (*James*)

All the specimens bred out were from larvæ found in bamboos

The type females of *W. aranoides* and *R. ceylonensis*, and the type male and female of *S. fusca*, are in the British Museum, the first from Taipang, Perak, Straits Settlements (*Wray*), the second from Ceylon (*Green*), and the last from the Malay Peninsula (*Leicester*). The type male of *S. mornata* is in the Indian Museum, from Dawna Hills, Burma, iii 1908 (*Annandale*).

### ***Rachionotomyia affinis* Edw**

*Rachionotomyia affinis*, Edwards, 1913, *Bull Ent Res*, Vol IV, p 241, nom nov

*Phonomyia coeruleocephala*, Theobald, 1910, *Mon Cul*, Vol V, p 577 (nec *Colonemyia coeruleocephala*, Leicester, 1908, *Cul Malaya*, p 233)

This may be distinguished from *R. aranoides* (Theo) by the characters given in the key. The male genitalia of the two species are very similar in structure.

Specimens have been examined from the following places—

Bombay Deccan—Belgaum, viii 1921, larvæ from tree-hole (*Barraud*)

Nilgiri Hills—x 1915 (*Khazan Chand*)

North Coorg—Santi Kappa, v 1914 (*Bambridge Fletcher*)

Types of both sexes are in the British Museum, from Hakgala, Ceylon, iii 1907 (*Green*).

### ***Rachionotomyia similis* (Leic)**

*Colonemyia similis*, Leicester, 1908, *Cul Malaya*, p 235

This species has not been noted as occurring in India before. There are a few specimens in the Malaria Survey of India collection, Kasauli, which agree very closely with Leicester's original description. The mesonotal scales are hair-like and of a greenish-brown or yellowish-brown colour, and there are no bristles on the disc. On the anterior part of the vertex there are scales of a deep blue colour forming a median patch wider in front than behind. All the femora are marked with two silvery spots, the medial one on the hind femur not always very distinct, being sometimes confluent with a silvery streak running from the base. Abdomen marked with large lateral silvery patches extending on to the dorsum to form narrow apical bands, scutellar scales flat and bronzy-green.

Male genitalia lobes of ninth tergite wide and armed with a number of flattened spines or leaflets (Plate LXXXVI, fig 8), clasper enlarged on the apical half and comparatively short (Plate LXXXVI, fig 7).

67	61	720	359	34	254	08	79	00	18
74	17	85	Nil	258	Trace	81	Trace	00	04
75	15	18	Nil	314	Nil	18	Nil	20	00
79	33	220	Nil	179	31	179	Trace	190	10
82	301	532	68	123	250	74	137	51	03
90	22	75	Nil	266	Trace	65	Trace	73	10
91	269	64	Trace	301	Trace	55	Trace	67	09
95	19	1'0	Nil	329	Trace	0'9	Trace	Trace	01
96	314	485	Trace	164	280	26	140	Trace	39
108A	227	585	Trace	112	277	196	95	Trace	17
109A	148	465	Trace	107	165	208	62	150	30
111	52	501	Nil	08	84	386	05	336	26
112	19	62	Nil	282	Trace	54	02	62	06
113	65	35		294	Trace	34	Nil	36	01
114	260	706	Trace	56	350	235	113	Trace	08
115	37	182		182	14	166	Nil	224	02
117	106	22	Trace	328	Nil	21	Nil	34	01
124	43	375	Trace	41	18	340	07	430	10
126	13	20	Nil	317	Nil	17	Nil	Trace	03
128	24	97		297	Trace	54	Trace	70	43
130	28	420	41	08	14	327	08	427	30
141	63	430	Trace	11	26	377	Trace	431	27



No member of this genus has previously been noted as occurring in India, although three Oriental species have been described from the Philippine Islands and the Malay Peninsula. One of these *H malayi* Leic has been recorded from Colombo, Ceylon (*James*)

The Malania Survey of India collection, Kasauli, contains one female specimen of a new species described below, bred by Sub-Assistant Surgeon J D Baily, I M D, after whom it is named

There is one character which distinguishes the species belonging to this genus from other mosquitoes, viz, the peculiar form of the plume scales on the wings. These scales are narrow and emarginate at their tips and appear as though the sides were prolonged into minute spines beyond the end of the scale. The venation also is characteristic of this and of two or three other genera of small forms (*Uranotaenia*, *Harpagomyia*, and some species of *Topomyia*), the commencement of vein 2, the base of the bifurcation of vein 5, and the tip of vein 6, lie one below the other and a line joining these points forms a right angle with the costa

All the Oriental species are of small size, and are probably frequently overlooked on this account

### ***Hodgesia bailyi* sp. n**

This is evidently allied to *H malayi* Leic but differs in the coloration of the mesonotum and pleuræ, and possibly in other details

#### **Description of female**

Head flat scaled, a broad band of silvery scales along the eye margins, widest in the middle where it occupies about half the dorsal surface, behind the straight posterior margin of this band the head is covered with black scales continued to the nape, apparently no upright scales present, tori brown, flagellar segments of antenna and hairs dark brown, clypeus dark brown, palpi and proboscis brownish-black, the former minute, the latter widened on the apical half when viewed from above

Thorax integument of the mesonotum pale yellowish on the anterior half, sparsely covered with black hair-like scales and bristles, a large brownish-black oval area immediately over and in front of each wing base (in *H malayi* the mesonotum is described as being 'uniformly reddish-brown' or 'ferruginous'), scutellum, and a small space on the mesonotum in front of it, yellowish, postnotum dark brown pro-epimeron, post-spiracular area, sub-alar knob upper part of mesepimeron and coxæ, yellowish, sterno-pleura and lower part of mesepimeron black, the upper part of the dark area overlaid with patches of flat silvery scales (in *H malayi* the pleuræ are described as being 'ferruginous'), two black pro-epimeral bristles, prothoracic lobes covered with flat silvery scales

Wings scales and venation typical of the genus. Wing length 2.5 mm

Legs tibiae and tarsi dark brown with a metallic bronzy sheen, which may cause them to appear light when viewed in certain positions, fore femora light on about the basal half, otherwise dark brown, mid pair dark brown anteriorly,

sodium, and may often be due to stones containing sodium (or potassium) silicate and thus under the action of hydrochloric acid changing into silicic acid and a sodium salt. The sodium salt would go into solution and the silicic acid would be weighed as insoluble ash. So far as we could determine, the insoluble ash is silica.

In addition to the above constituents, in some stones carbonates were present and when qualitative tests indicated their presence, the  $\text{CO}_2$  was determined in a Schroedter apparatus. The results were as follows —

Stones	65	2.0 per cent
	111	2.0 „
	114	1.4 „
	20	0.6 „
	60	1.0 „
	86	1.6 „

*The nature of the nitrogen compounds in stones*

Most of the nitrogen is in the form of uric acid (or some other purin compound of like composition) and in all the stones with more than 5 per cent of nitrogen some of it is in this form. This is shown by all the stones with any considerable content of nitrogen giving the murexide test, and also by the factor by which the nitrogen has to be multiplied to make the analyses add up to 100. The factors which the total nitrogen has to be multiplied by are —3 for uric acid, 1.3 for ammonium and 6.25 for protein. Taking all the analyses, we have as mean figures —

Ash	.	26.4
Oxalates	..	11.8
		<hr/>
Total	..	38.2
		<hr/>
Difference from 100	..	61.8
		<hr/>
Total nitrogen		19.1
		<hr/>
Difference/Total nitrogen		3.24

which, if only uric acid and protein were present, would correspond to 93 per cent uric acid and 7 per cent protein, and in most of the stones this seems to be approximately the case (*cf* Table II)

## EXPLANATION OF PLATE LXXXVI

Camera lucida drawings showing structural characters in *Mucidus*, *Ficalbia*, and *Rachnonotomyia*

Figs 1, 2, and 5 to 10, drawn to the scale shown under Fig 5

- |       |                                    |   |
|-------|------------------------------------|---|
| Fig 1 | <i>Mucidus langer</i>              | Harpago of male genitalia (flat preparation)  |
| „ 2   | <i>M scataphagoides</i>            | Ditto   |
| „ 3   | <i>Ficalbia minima</i>             | Torus and first and second flagellar segments of antenna of female                              |
| „ 4   | <i>Ficalbia minima</i>             | Proboscis and palp of male  |
| „ 5   | <i>Rachnonotomyia aranoides</i>    | Side view of male genitalia from the outside, showing one lobe of the ninth tergite to the left |
| „ 6   | <i>R powelli</i> var <i>indica</i> | Side-piece and clasper of male genitalia from the inside (flat preparation)                     |
| „ 7   | <i>R similis</i>                   | Clasper of male genitalia (flat preparation)  |
| „ 8   | <i>R similis</i>                   | One lobe of the ninth tergite of male genitalia (flat preparation)                              |
| „ 9   | <i>R powelli</i> var <i>indica</i> | Ditto   |
| „ 10  | <i>R aranoides</i>                 | Ditto   |

TABLE II

Stone number.	Nitrogen as per cent of dry stone in the form of	
	NH <sub>4</sub>	Protein
2	3 8	
4		0 6
5		1 0
18	0 0	
19	5 2	
21		1 0
26		0 8
60		1 0
83		1 2
85		2 1
86		0 7
102		1 1
104		2 4
121		1 8
123	4 5	
137		0 7
149		0 7
152		1 0
163		0 6
172		0 8
173		0 2
176	10 0	
8		1 0
10		0 8
13	4 4	



*General composition of the stones*

The chief constituents of the stones are uric acid or urates, phosphates and oxalates combined with calcium and magnesium

Using the capital letters N, P, C, M and O to mean the stones with more than the average nitrogen, phosphates, calcium, magnesium and oxalates, the associations of these five constituents are shown in Table III

TABLE III

*Showing the principal classes of the associations between the main constituents of the 100 stones*

N	53	NP	2	NPC	0	NPCM	0
P	29	NC	2	NPM	2	NPCO	0
C	38	NM	4	NPO	0	PCMO	5
M	17	NO	5	NCM	0	NCMO	0
O	37	PC	19	NCO	1	NPMO	0
		PM	14	NMO	0	NPMCO	0
		PO	13	PCM	11		
		CM	13	PCO	13		
		CO	31	PMO	5		
		MO	5	CMO	5		

Association coefficients			
NP	-0.94	PM	+0.92
NC	-0.98	PO	+0.23
NM	-0.65	CM	+0.77
NO	-0.91	CO	+0.95
PC	+0.68	MO	-0.20

The association coefficient used is the one called Q in Udney Yule's *Theory of Statistics*, 1911, p 38

The association coefficients shown in the tables are of interest in showing —

1 The nitrogen is negatively associated with all the other four constituents, that is to say, that for the most part the nitrogen exists in the form of some nitrogen compound uncombined with calcium, magnesium, etc—presumably uric acid

2 The phosphates exist as calcium and magnesium phosphate

3 The oxalates as calcium oxalate

4 The magnesium exists as magnesium phosphate

5 That calcium and magnesium commonly go together

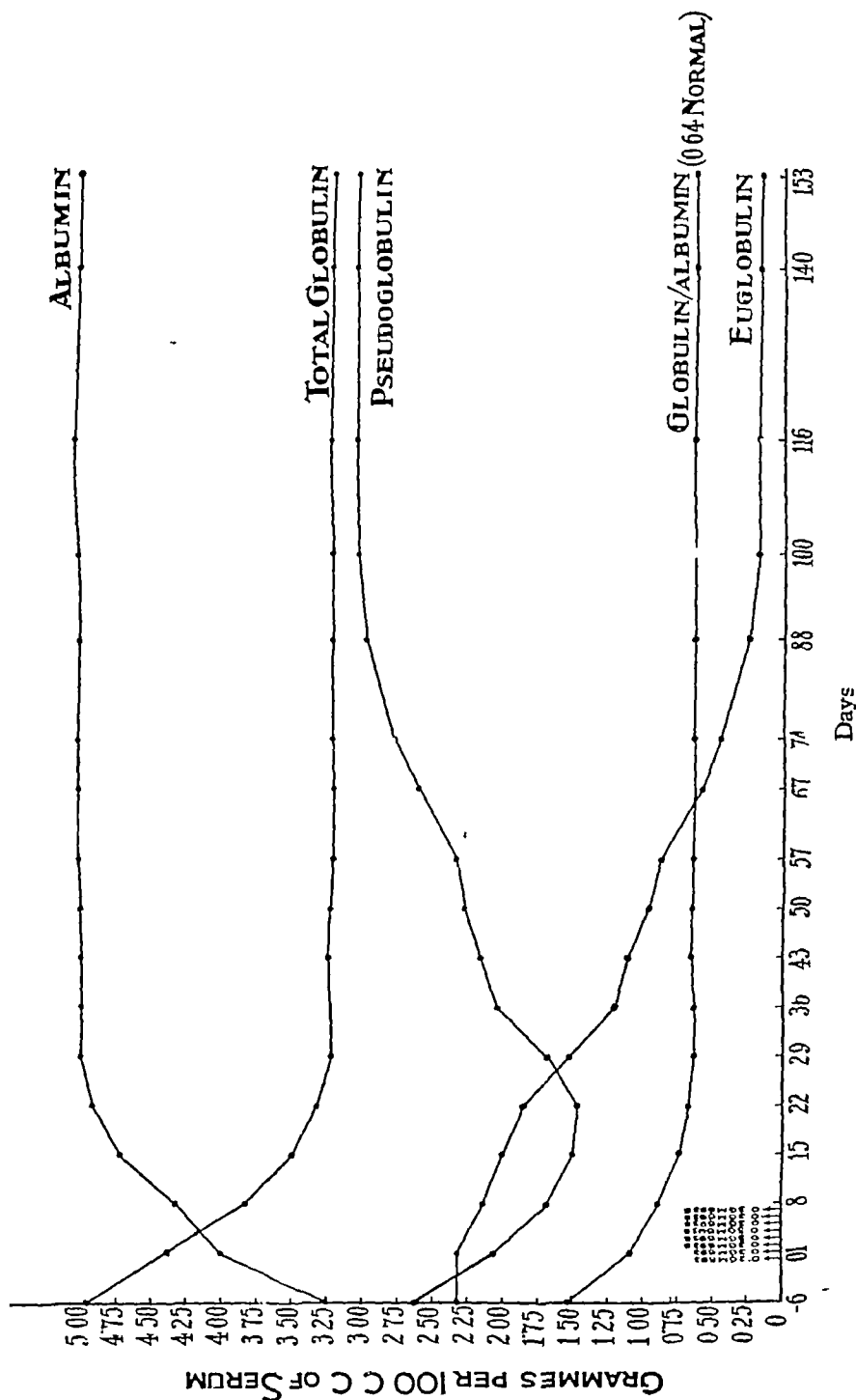
6 That stones tend to be on the one hand uric acid stones or, on the other hand, to consist of magnesium phosphate and calcium phosphate and oxalate. Those stones in which both of these two classes of constituents are present are generally layered, the two classes being precipitated at different times. The conclusion seems irresistible that the factor which determines which of the two classes shall be thrown down is the acidity or alkalinity of the urine.

6 Treatment of 'resistant' cases by 693 B

7 Atypical cases

The graphs which follow illustrate the effect produced upon the serum proteins by the variations in treatment in the various clinical types detailed

GRAPH 1



3 In some stones small amounts of ammonia, carbon dioxide, sulphate and silica (probably as sodium silicate) are present in combination

4 Stones composed wholly of one substance are rare—the notable exception being stones composed almost entirely of uric acid (9 per cent of the stones analyzed)

5 The most common form of stone is a mixture of uric acid or urates and oxalates (38 per cent of the stones analysed)

6 In 83 per cent of the stones some uric acid or urates were present

7 Stones which consist of uric acid or urates mixed with phosphates and oxalates (one or both) are often layered indicating that the uric acid is deposited at different times to the other constituents

8 No particular kind of stone seems to be peculiar to any special part of India so far as the numbers analysed show

#### REFERENCES

FOLIN and WU (1919)

*Jour Biol Chem*, XXXVIII, p 100

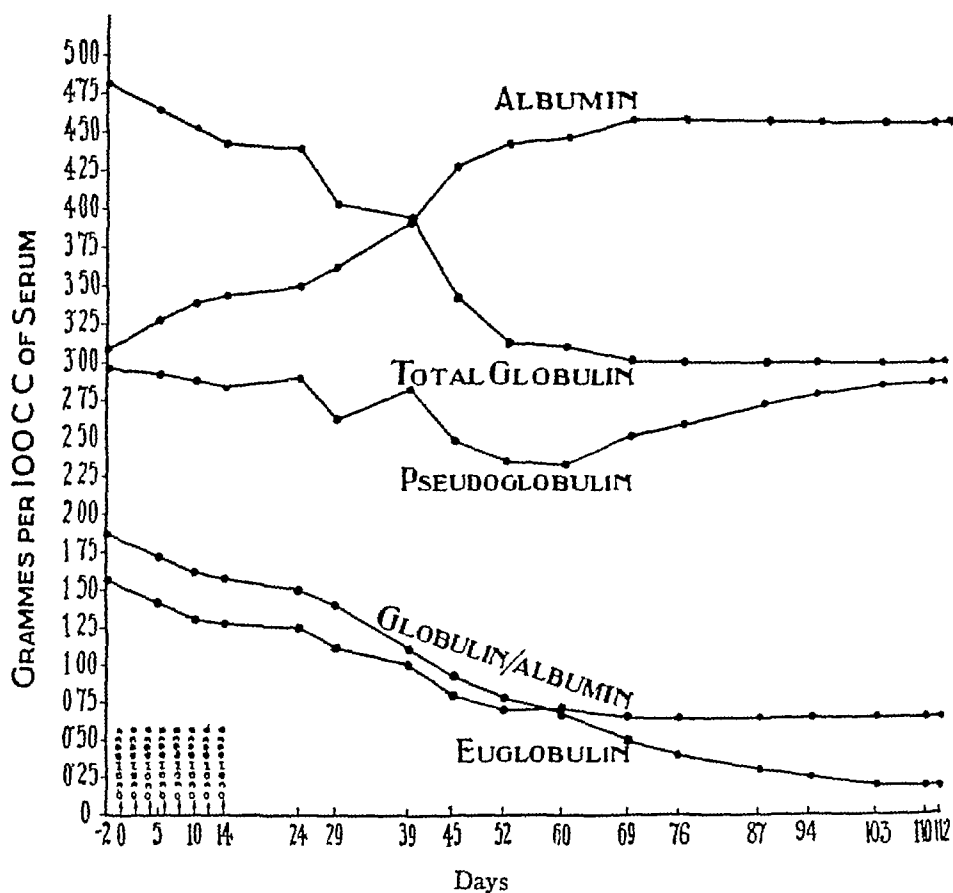
BENEDICT (1922)

*Ibid*, LI, p 187



slower than in the standard graph, being only entered on about the 52nd day, i.e., approximately twice as long as in the standard graph. The initial sudden fall of the pseudoglobulin being absent, the pseudoglobulin graph does not intersect the euglobulin curve, but the onset of the second stage is seen as

GRAPH 2



the point where the pseudoglobulin and euglobulin curves diverge. Eight alternate daily injections of 693 B then produce a much slower protein response than the concentrated course of the same total dosage. The temperature of this patient reacted satisfactorily becoming normal on the 16th day. His weight increased steadily. His spleen culture was positive on the 28th day, but was negative on the 56th day.

This patient received exactly the same treatment as the previous one. Graph 3 is accordingly of exactly the same type as Graph 2, and calls for no separate description. We may take these two Graphs 2 and 3 as typical of the serum changes produced by alternate daily injections of 693 B (total dosage 2.3 grms) on an average well-established case of kala-azar with high total globulin and euglobulin values.

**Mucidus scataphagoides** Theo

Theobald, 1901, *Mon Cul*, Vol I, p 277

This is a common and widely distributed species in India and may be distinguished from *M. laniger* (Wied) by the presence of a ring of white scales in the middle of the first hind tarsal segment, this segment being also distinctly shorter than the tibia

There is some variation in the markings of the proboscis and wings. In some specimens there is very little white scaling on the former, this organ being mainly yellow-scaled. The amount of dark brown and yellow scaling on the wing is variable, in some specimens the costa is almost entirely yellow, in others it is dark-scaled for some distance from the base, but there is nearly always a rather large yellow-scaled area towards the apex of the wing which extends on to the first vein.

The harpago of the male genitalia is shorter, and the blade wider in this species than in *M. laniger* (compare Plate LXXXVI, figs 1 and 2)

Distribution in India —

Rajputana — Nasirabad, 22 viii 1927 (Central Malaria Bureau records)

Punjab — Amritsar and Lahore (*Christophers*); Ferozepore (C M B records), Karnal district, vii 1928 (*Sinton*), Sialkot, 1912 (C M B records), Jhelum, 1927 (C M B records)

Western Himalayas — Kasauli, 5 ix 1923 (*Barraud*)

Delhi Province — Delhi (*Hodgson*, *Christophers*, and *Major Wooly*), 10 x 1927 and 7 xi 1927 (*R. Semor White*)

United Provinces — Saharanpur, 22 ix 1927 (*Barraud*), x 1927 (*Covell*), Cawnpore, 28 viii 1913 (*F M Howlett*), Bareilly (C M B records), Moradabad (*Major Close*, *IMS*) [Theobald]

Central Provinces — Kamptee, 1926 (C M B records)

Bihar and Orissa — Pusa, various dates (*Bainbrigge Fletcher*, *H N Sharma*, and *Shaffi*), Saran (*Mackenzie*), Chatra and Kodarma, Hazaribagh district, 10—13 ix 1928 (*Dr Korke*), Purneah, 5 viii 1907 (*C Parva*)

Bengal — Bauria, 17 viii 1907 (*Tyrie*) [Theobald], Damukdia Ghat, Eastern Bengal, 22 viii 1907 [Theobald]

Burma — Bhamo, 1925 (*Feegrade*), Mandalay, 1912 (*Major Brown*), Myingan (*Watson*) [Theobald], Maymyo (*Bennett*) [Edwards' MSS]

Madras — Guindy, 1925 (*King Institute*), Anantapur, 1926 (C M B records)

Ceylon — (*Green*) [Theobald]

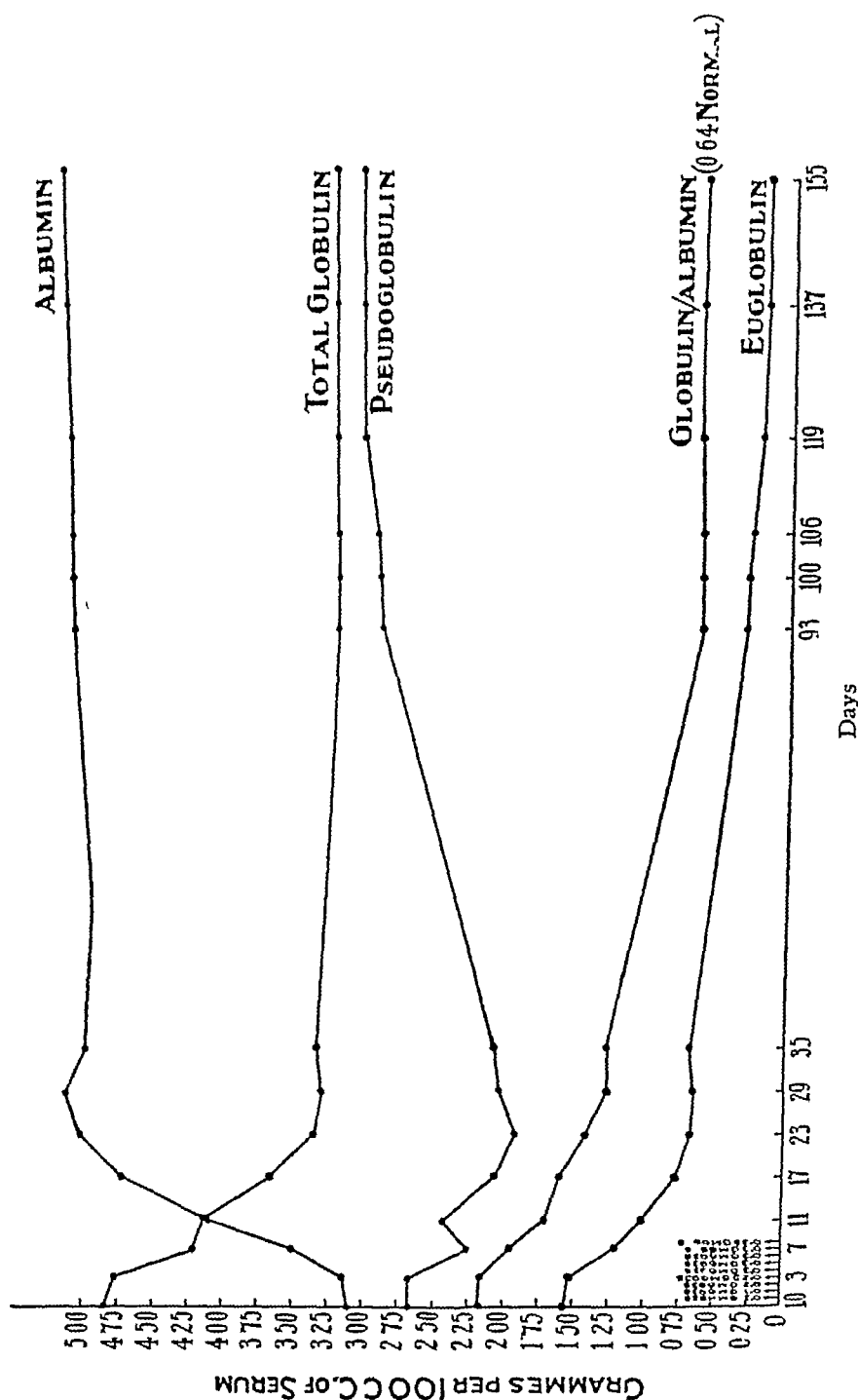
**Mucidus laniger** (Wied)

*Culex laniger*, Wiedemann, 1821, *Dipt Exot*, p 9

This species is distinguished from *M. scataphagoides* by the absence of a medial white ring on the first hind tarsal segment, this segment being about the same length as the tibia and not distinctly shorter than in the other species

0.3 gm were given, and finally one of 0.35 gm to make up the usual total dosage of 2.3 grms. The duration of the fever in this case was about three

GRAPH 4



years. The clinical response to treatment was good, the temperature becoming normal by the 9th day. On admission his spleen was 9 inches below the costal

This is a small dark species resembling, in the female, some species of *Ficalbia*, owing to the large wing scales, but the anterior fork-cell is short and only about half the length of the stem

There is one female in the Malaria Survey of India collection, Kasauli, from Santragachi, Howrah, Lower Bengal, 4 viii 1926 (*R Senior White*), one female in the collection of the Agricultural Research Institute, Pusa, from Dacca, xii 1911 (*S K S*) One male and one female have been recorded from Kierpur, Purneah district, Bihar (*C Parva*) [Edwards' MSS]

The type male and female of *L. minima* came from Carandagan, Mindanas, Philippine Islands, 19 i 1906 (*W H Duncan*), and are presumably in the Army Medical Museum, Washington *C hybrida* was described from one male from Kuala Lumpur (*Leicester*), and from other males and one female from Singapore (*Finlayson*), the types of these and of No 1 undetermined species, from Kuala Lumpur (*Leicester*), are in the British Museum

### ***Mimomyia chamberlaini* Ludl**

*Mimomyia chamberlaini*, Ludlow, 1904, *Can Ent*, Vol XXXVI, p 297

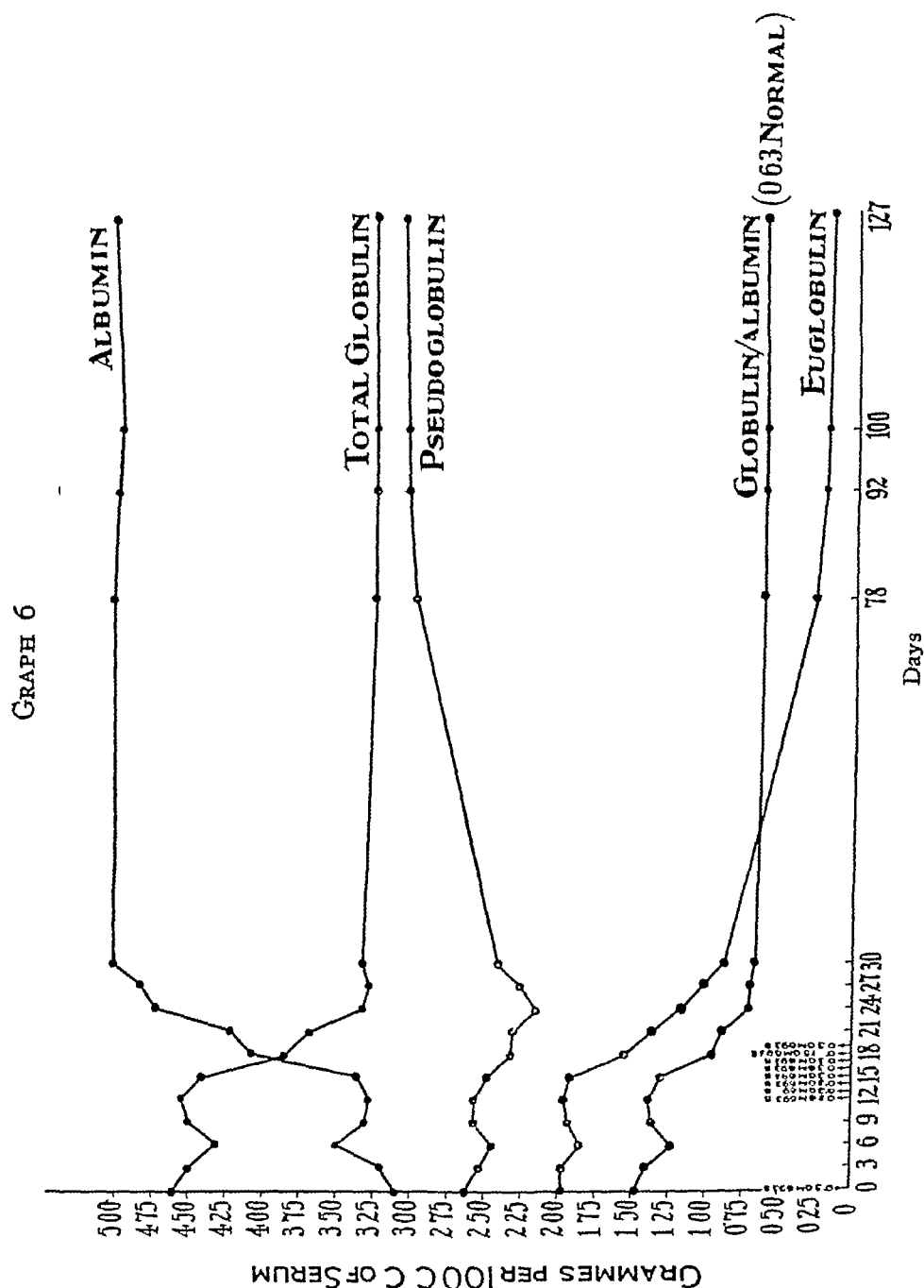
*Radioculex clavipalpus*, Theobald, 1908, *Rec Ind Mus*, Vol II, p 295

This species is widely distributed in India and may be fairly easily recognised by the presence of yellow scales along the sides of the thorax, contrasting with the dark brown shiny dorsum of the mesonotum, there are pale rings on the tarsi and the last segment of the hind tarsi is entirely pale The only other Indian mosquito with which this might be confused is *Aedes (Banksimella) lincatopennis* Ludl in which the thorax is marked in rather a similar manner, but in that species the tarsi are entirely dark In the typical form of *M chamberlaini*, as known in India, the dorsum of the abdomen is entirely covered with dark violet scales and the dark parts of the legs are of the same colour

#### **var n *intermedia*.**

There is a form occurring in Assam, which may be a distinct species, having large lateral yellow patches on the dorsum of the abdomen, uniting in some specimens to form basal bands The genitalia of the males of this variety show some small differences, the clasper being distinctly shorter than in the males of the commoner form, but this difference, like the markings, may only be due to local variation The larva of this variety has not yet been obtained The original description of *M chamberlaini* by Miss Ludlow (made from a single male specimen) appears to apply better to this variety than to the form found more commonly in India, as the abdomen is described as having 'very large basal lateral light spots forming an almost continuous lateral yellowish stripe' Possibly there are two very similar species, one occurring in Assam and the Philippines (*chamberlaini*), and one distributed over the larger part of the Indian sub-continent (*clavipalpus*)

Graph 6 also shows the effect of a single injection of 693 B. The changes are again quite typical. The downward dip of the pseudoglobulin is in this case associated with a rise of the albumin. Relapse soon followed as shown by the hump on the globulin/albumin ratio curve. A full course of treatment was



commenced on the 12th day with the typical favourable response, the second stage commencing on the 24th day. This case was followed to serological cure on the 127th day with the euglobulin down to normal (0.16 gm per 100 cc

The three species found in India belong to three different groups, which were formerly treated as separate genera. The points of distinction between these are given in the following brief descriptions.

### ***Ficalbia fusca* (Leic)**

*Dasymyia fusca*, Leicester, 1908, *Cul Malaya*, p. 102

This species belongs to the *Ingramia* group, and is distinguished from the other two species as follows —Wing scales dark, tarsi without any pale rings or spots, abdomen entirely dark dorsally, palpi of the male about two-thirds the length of the proboscis, first flagellar segment of antenna of female very slightly longer than the third.

It appears to be very rare and I have only seen one female from the Andaman Islands, ix 1911 (*Christophers*)

The type male and female are in the British Museum from the Malay Peninsula (*Leicester*)

### ***Ficalbia luzonensis* (Ludl)**

*O'Reillia luzonensis*, Ludlow, 1905, *Can Ent*, Vol XXXVII, p. 101

*Etiroleptomyia completiva*, Leicester, 1908, *Cul Malaya*, p. 178

This represents the *Etiroleptomyia* group and is distinguished by having the wings speckled with light and dark scales, the tarsi mainly yellow with dark spots, abdomen with a median dark stripe on a yellow ground, palpi of the male nearly as long as the proboscis, first flagellar segment of antenna of female about as long as the second.

I have only seen one specimen of this species from India, a male from Calcutta, x 1910 (*H N Sharma*). The Kasauli collection contains one pair from Colombo, Ceylon (*James*)

*O'R. luzonensis* was described from one female from Bayambang, Pangasinan, Luzon, Philippine Islands, caught 11 ix 1904 outside screens of screened house on a rainy night (*Chamberlain*), the type is presumably in the Army Medical Museum, Washington. The unique type male of *E. completiva*, from Singapore (*Finlayson*) is in the British Museum.

### ***Ficalbia minima* (Theo)**

*Uranotaenia minima*, Theobald, 1901, *Mon Cul*, Vol II, p. 262

*Mimomyia minuta*, Theobald, 1908, *Rec Ind Mus*, Vol II, p. 301 (male),  
1 c 1910, Vol IV, p. 30 (female)

This is the only species of the genus which appears to be fairly common in certain parts of India. As far as is known at present it is confined to Travancore, Bengal, Orissa, and Assam. It represents the true *Ficalbia* group in which the palpi of both sexes are quite short and the first flagellar segment of the antenna of the female is about three times the length of the second (Plate LXXXVI, fig 3). The wing scales are dark, tarsi marked with narrow pale rings, most distinct on the hind legs, and the abdomen marked with transverse pale bands.

total globulin and rise in the albumin. Practically no change occurred in the pseudoglobulin, most of the fall being in the euglobulin. The protein progress was normal up to the 10th day, after which there was some tendency to relapse which did not proceed very far. The patient was watched very closely and as he was doing well was left without further treatment. On the 26th day there was a definite turn downwards of the total globulin, and from this point the graph progressed normally to reach serological cure on the 110th day, i.e., within the usual four months required for serological cure with concentrated courses of treatment. It will be noticed that the onset of the second stage was delayed to about the 59th day. This graph indicates that this patient's resistance with the aid of two doses of 693 B was just able to effect a cure. The appearance of this graph suggests that we hit off the absolute minimum of treatment which would have cured this patient. We consider this graph of great importance, as no instance of cure of kala-azar by means of two injections of 693 B has yet been reported, and it further brings out one of the most important applications of the graph test, viz., the prevention of over-treatment. This graph well illustrates a very curious feature which is very constant, viz., that no matter whether the treatment given is much or little, provided that it is sufficient for cure, the period required to reach serological cure is always about the same (4 months), even though with very low dosage, such as was given in this case, the second stage does not begin till the 59th day. The body appears to possess a curious power of compensating by increased speed in the second stage for slowness in the first.

Clinically this was a well-established case with nine months fever of insidious origin. The temperature fell to normal on the 14th day, and remained almost normal for six weeks before discharge. The spleen was 7 inches below the costal margin on admission and only just palpable on discharge.

Graph 8 also shows the effect of two injections of 693 B. The early changes were quite typical, with a big drop in the pseudoglobulin in this instance.

This graph shows, as did the last, the tendency to relapse. From the 10th day the globulin gradually but steadily went back until on the 30th day it was considered advisable to recommence treatment. A full course was then given. The patient entered the second stage on the 45th day and reached serological cure on the 107th day. Two injections evidently just failed to produce a cure in this case. As in the last graph, it will be seen that in consequence of the inadequate first course the patient took much longer than usual to enter the second stage, yet the time of serological cure was not thereby lengthened. In a previous paper the senior writer stated the belief that a relapsing globulin/albumin ratio indicates a refractory case, or as a corollary incomplete treatment of an average case. Graph 8 shows this very clearly.

The history in this case was of six months fever with a malarial onset. The patient did not show very marked clinical improvement in hospital. His weight remained unchanged and at the time of discharge the temperature was still up to 99° daily, but his spleen which was 6 inches below the costal margin before treatment was only just palpable at the time of his discharge.

larvæ may be found living in hollow bamboos and tree-holes during the rainy season. The commonest species is *R. aranoides* (Theo), and this may be found near villages and cantonments, when there are suitable breeding-places in the vicinity.

Synoptic table for the identification of the Indian species of *Rachionotomyia*

1	Femora spotted	2
	Femora unspotted	3
2	Prothoracic lobes covered with flat silvery scales	<i>similis</i>
	Prothoracic lobes with a row or two of small flat brown scales	<i>powelli</i> var <i>indica</i>
3	Pro-epimera and pleuræ covered with white scales, a narrow blue border to the eye margins	4
	Pro-epimera covered with brown scales, silvery scales on the pleuræ, a wide deep blue border to the eye margins	<i>affinis</i>
4	Light scales forming a lateral straight border to the abdominal tergites for the length of the abdomen	<i>aranoides</i>
	Light scales forming lateral triangular patches, or a serrated border, to the abdominal tergites for the length of the abdomen	<i>aranoides</i> var <i>serrata</i>

### *Rachionotomyia aranoides* (Theo)

*Wyeomyia aranoides*, Theobald, 1901, *Mon Cul*, Vol II, p 24/

*Rachionotomyia ceylonensis*, Theobald, 1904, *Jour Bomb Nat His Soc*, Vol XVI, p 248

*Squamomyia inornata*, Theobald, 1910, *Rec Ind Mus*, Vol IV, p 28

*Skeiromyia fusca*, Leicester, 1908, *Cul Malaya*, p 248

The dark greyish-brown mesonotal scales of this species are distinctly broad, and there are no bristles on the disc, which gives the thorax a very smooth appearance. The blue scales forming a border to the eyes are best seen by viewing the insect from the front, or from the side with the head directed towards the light, the white scales which almost entirely cover the pleuræ are not brilliantly silvery as is the case in the allied species *R. affinis*.

**var n *serrata*** In this variety the white scales forming a lateral border to the abdomen do not form a straight line, as in the type form, but are arranged in triangular patches, the widest part of the triangle being at the base of each segment. I have not been able to detect any other differences in markings or structure.

Male genitalia lobes of ninth tergite with three or four strong spines (Plate LXXXVI, fig 10). This is also the case in *R. affinis*. Plate LXXXVI, fig 5, shows a side view of the genitalia.

Specimens have been examined from the following places —

Bombay Deccan — Nagargali, 13 viii 1921 (*Barraud*)

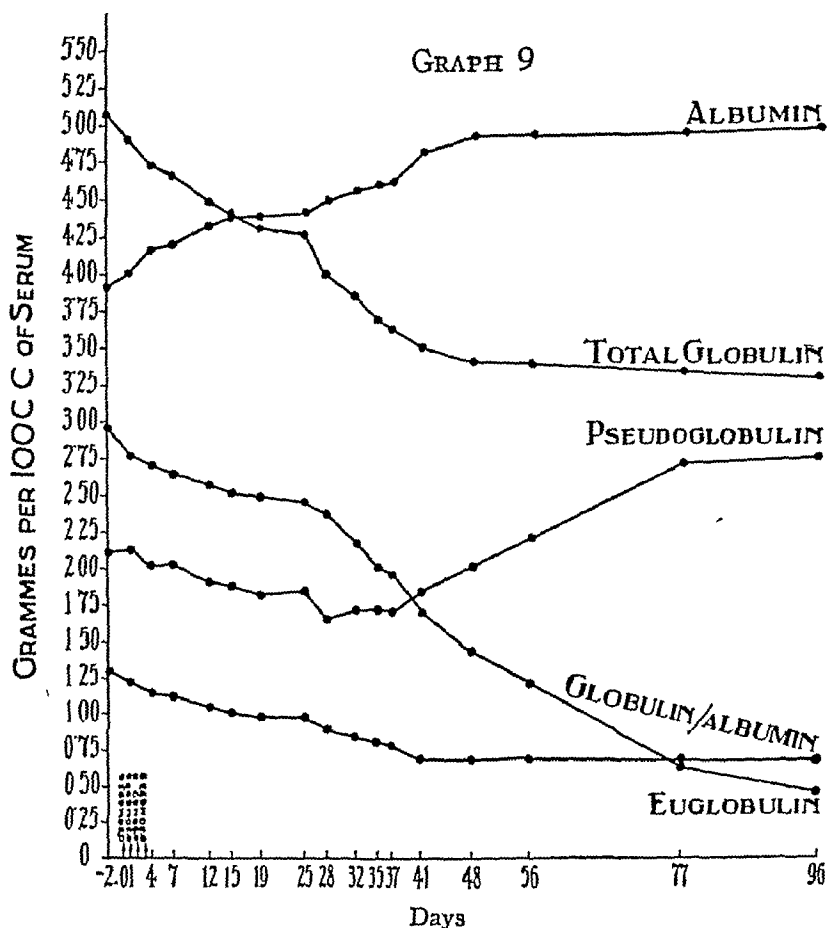
North Kanara — Kadra, ix 1921 (*Barraud*), Yellapur, x 1921 (*Barraud*)

Nilgiri Hills — λ 1915 (*Khasan Chand*)



stage on the 41st day, and with the sharp upward turn of the pseudoglobulin from that point the prognosis is excellent

In this case before treatment the euglobulin was higher than the pseudoglobulin. The euglobulin here reached the exceptionally high figure of very nearly



3 grms per 100 c c of serum. This evidently offered no obstacle to cure even by a half course of total dosage 11 grm.

Clinically the progress was favourable. The temperature fell to normal on the 5th day.

Graph 10 shows a type of case in which the pseudoglobulin was much higher than the euglobulin, yet it fell only slightly under treatment. The euglobulin fell gradually. The onset of the second stage is not so clearly discernible in this case, but it commenced about the 54th day. The case is progressing well towards serological cure, and it is evident that four doses of 693 B were quite sufficient. This is the more interesting as this case was classed as 'resistant,' a previous course of treatment given outside having failed to effect a cure.

The history in this instance is about six months fever of malarial onset. The temperature became normal on the 8th day afterwards rising occasionally to

I have examined specimens from —

Eastern Himalayas — Sureil and Mungpoo, x 1922 (*Barraud*)

North Bengal — Sukna and Marinbari Tea Estate, viii 1928 (*Sobha Ram* collr)

Co-type female specimens are in the British Museum, from Bukit Kutu, Malay Peninsula (*Leicester*)

***Rachionotomyia powelli* (Ludl) var n *indica*.**

*Uianotaenia powelli*, Ludlow, 1909, *Can Ent*, Vol XLI, p 235

There are a few specimens in the Kasauli collection which agree fairly closely with Miss Ludlow's description of '*Uianotaenia powelli*' (which is a species of *Rachionotomyia* previously known only from the Philippine Islands) but there are certain differences, mentioned below, which seem to indicate that the Indian form represents a variety, or possibly a distinct species

To the characters given in the key the following may be added — A broad area of flat metallic deep blue scales occupying about the anterior half of the dorsal surface of the head, and extending to the sides, the straight posterior margin of this area bordered with deep violet or brownish-black scales. Integument of prescutum and scutellum light brown, that of the scutum and postnotum dark brown, several pairs of long bristles on the disc. Mesonotal scales narrow and yellowish but there appear to be some darker ones intermixed (in the description of the type they are given as 'dark brown'), scutellar scales rather small, flat and dark brown. Integument of pleuræ of mesothorax black, an oval area of the same shade around the anterior spiracle, pleuræ of prothorax and metathorax and coxæ pale yellowish, a large area of brilliant silvery scales covering the larger part of the sterno-pleura and mesepimeron (description of type states 'pleura dark brown, heavily covered with white flat scales'). All the femora marked with two silvery white spots. Large silvery lateral patches on the abdomen not usually forming complete apical bands on the dorsum, the dark parts of the tergites brownish-black, appearing yellowish or bronzy when viewed in certain positions. Lobes of ninth tergite of male genitalia longer and narrower than in *R similis* (Leic) each armed with about six flattened bristles or leaflets (Plate LXXXVI, fig 9). Larger claw of fore leg of male simple.

Specimens have been examined from —

Assam — Golaghat (*Christophers*), Nongpoh, vii 1922 (*Barraud*)

North Bengal — Sukna, x 1922 (*Barraud*) and viii 1928 (*Sobha Ram* collr)

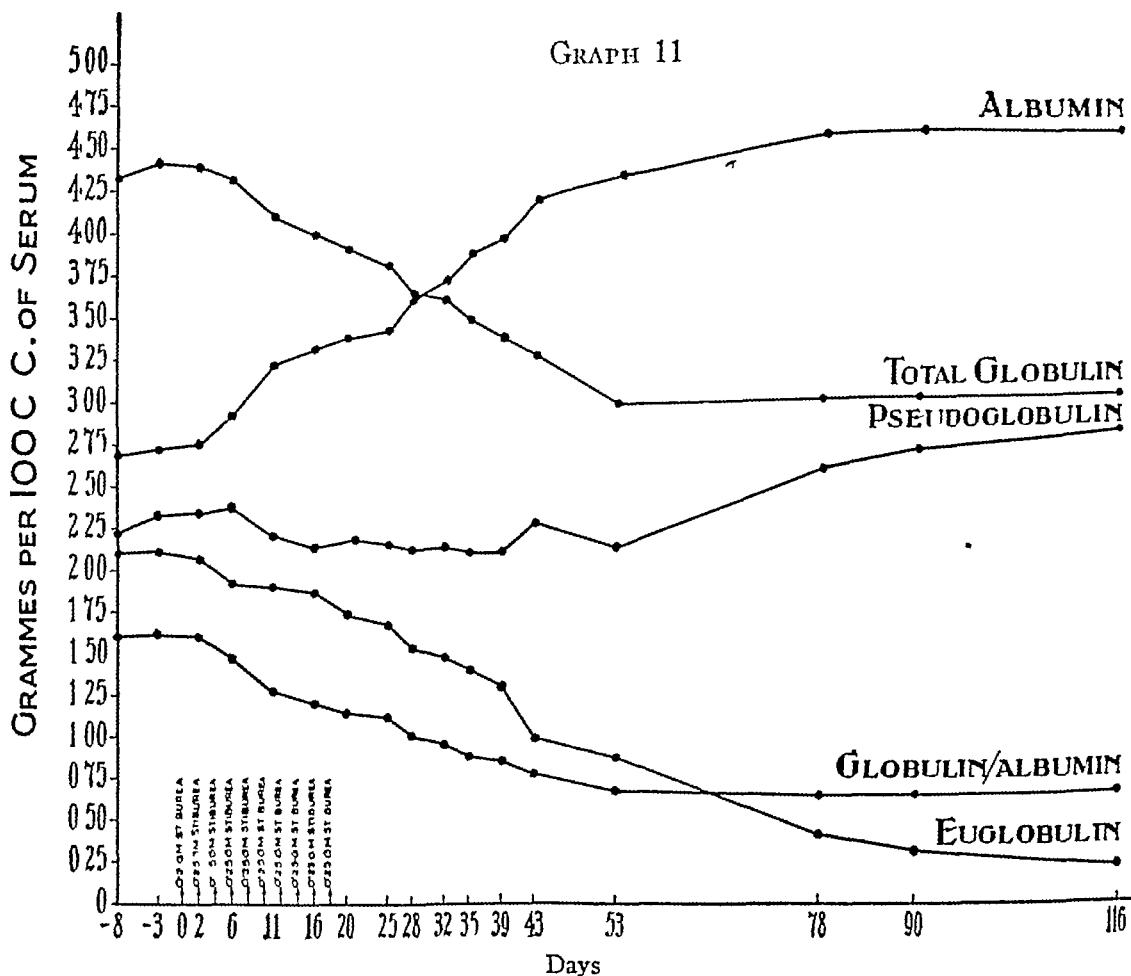
The type form was described from one female from Camp Wilhelm, Yayabas, Philippine Islands, 3 i 1909 (*W A Powell*), which is presumably in the Army Medical Museum, Washington

Genus HODGESIA Theo

Theobald, 1904, *Jour Trop Med*, January 15th, and *Mon Cul*, Vol IV, p 579, 1907

daily injections, a first injection of 0.2 gm and nine subsequent injections of 0.25 gm, i.e., a total dosage of 2.45 grms. The prognosis of this case is excellent, the second stage being reached on the 53rd day and serological cure being nearly effected by the 116th day. There is no sign here of the sudden drop of the pseudoglobulin.

This was a case of ten months fever of malarial onset. The temperature fell to normal on the 14th day. After a preliminary loss of weight, probably due to the disappearance of oedema, the patient gained 14 lbs.



Graph 12 is of exactly the same type as Graph 11 and the treatment is the same. This is of special interest in view of the fact that this patient was almost afebrile throughout, showing that there is no association between the protein behaviour, and the temperature response. This will have been noticed in the previous graphs where it is seen that the temperature usually falls to normal in very few days after the commencement of treatment, whereas the protein changes go on steadily for a long period.

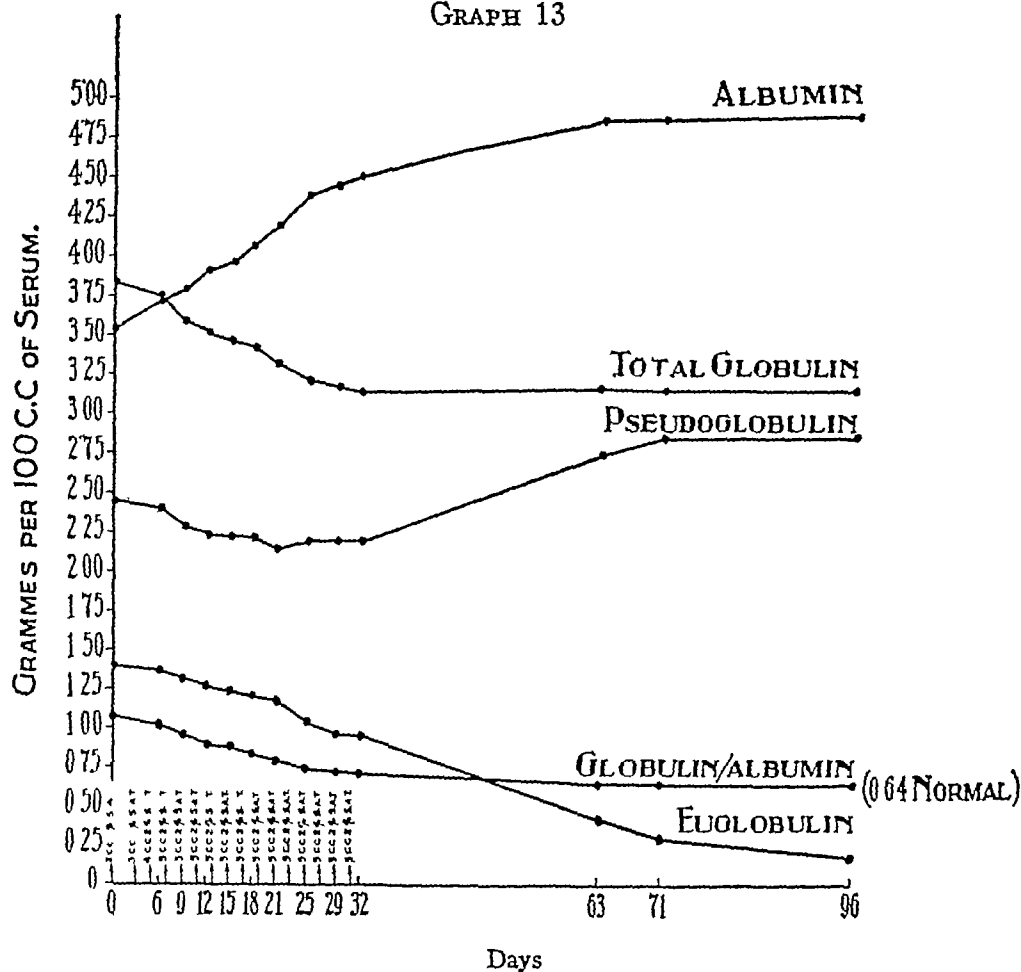
pale posteriorly and ventrally for the whole length, hind pair white on the outside for the whole length except for a few dark scales at the extreme tip, similarly marked on the inside except for a short dorsal brown streak to the knee

Abdomen entirely bluish-black with metallic lustre, very blunt at the extremity owing to the retraction of the terminal segments, cerci very small and completely hidden, only one chitinized spermatheca, as in the females of *Ficalbia minima* (Theo) and *Anopheles*

The type female is at present in the collection of the Malaria Survey of India, Kasauli, from Virajpet, Coorg, South India, vi 1927 (*J D Baily*)

globulin and the albumin relapsed. This was associated with the appearance of grave cedema of the legs and the patient died two days after the last observation on the graph. This patient had had no previous treatment. No other history was available.

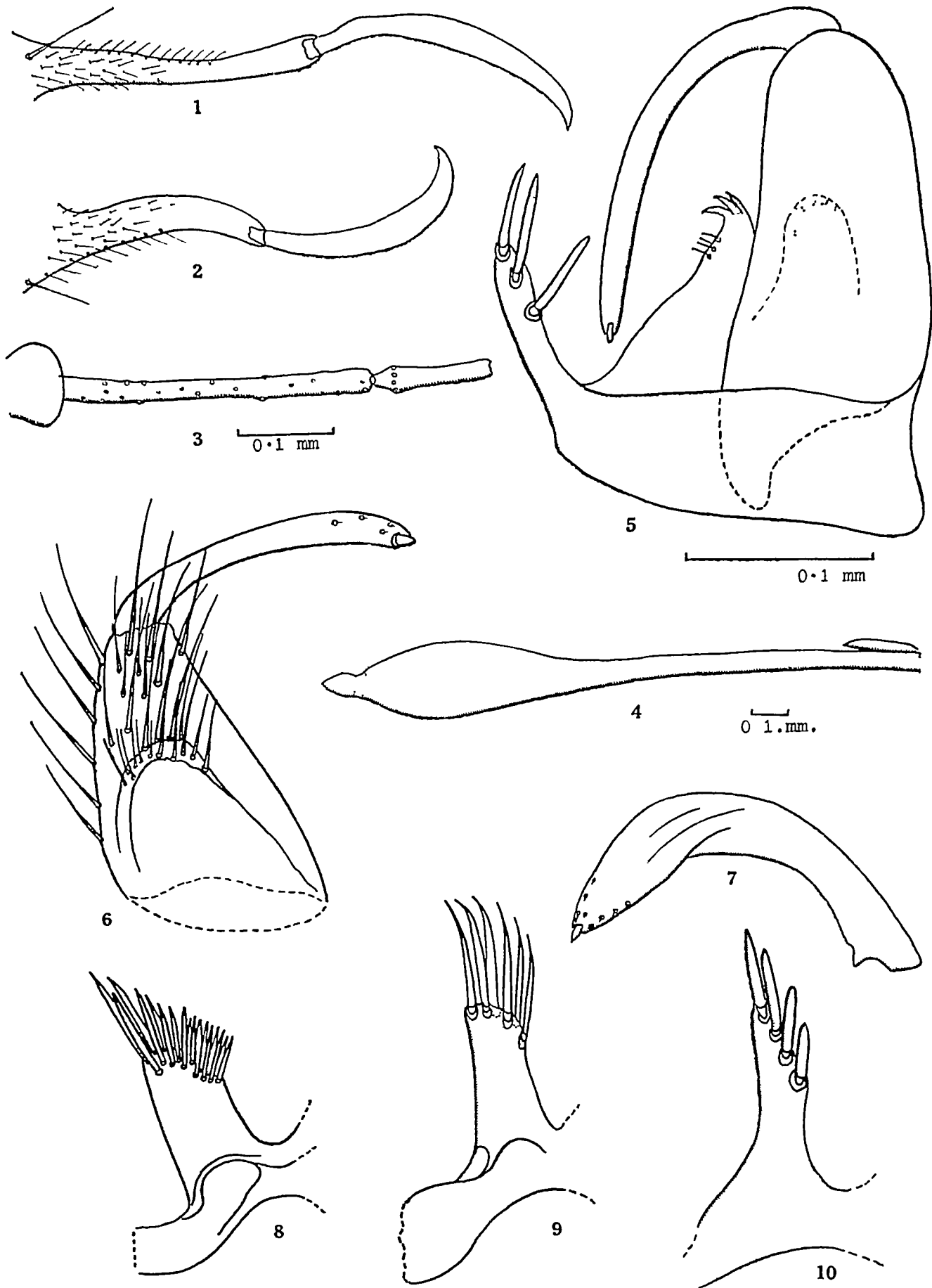
GRAPH 13



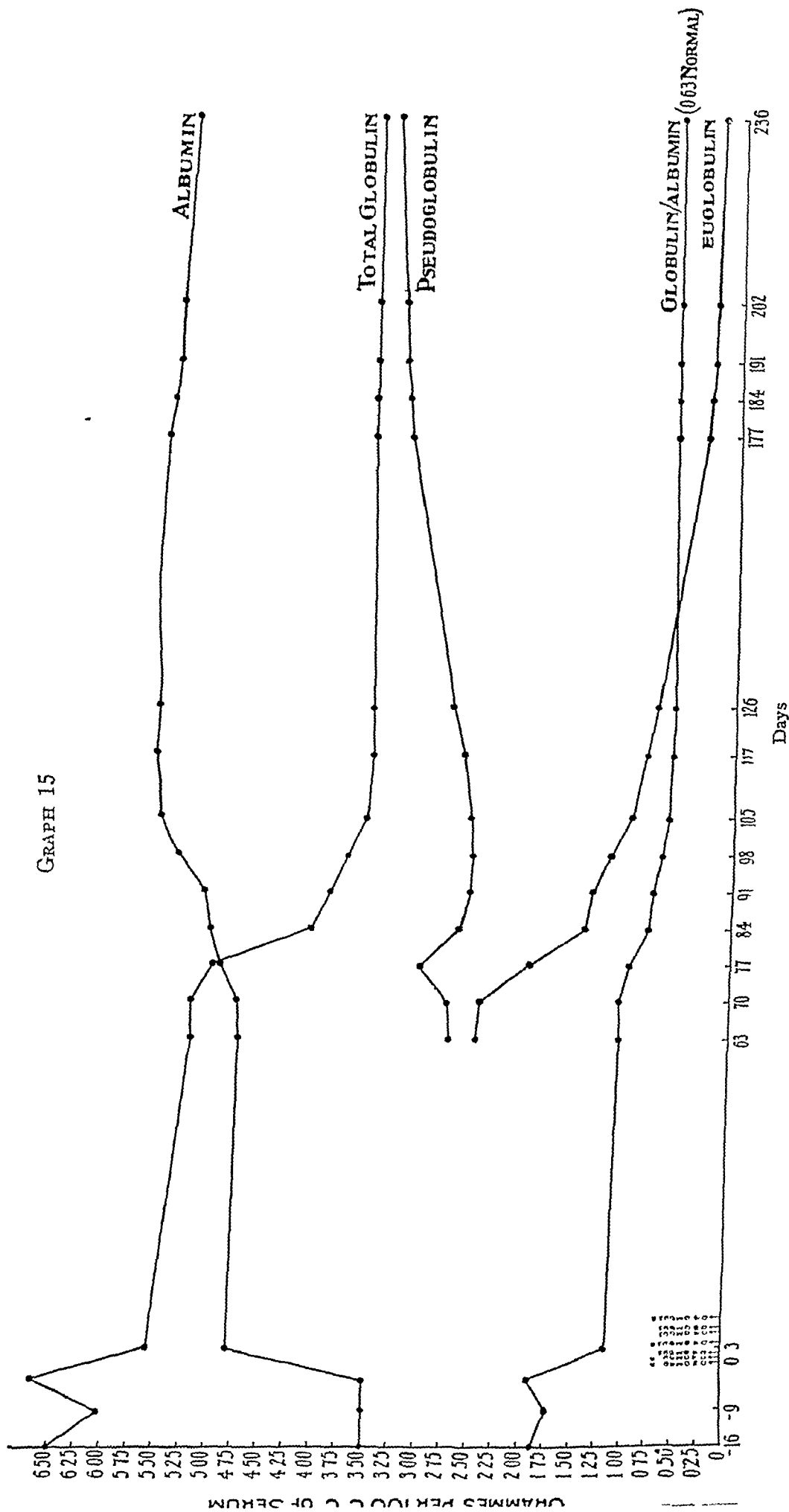
The protein changes produced by sodium antimony tartrate have not been studied in any detail, and Graphs 13 and 14 have been included solely to bring out the point that the trivalent antimony compounds cause essentially the same protein changes as those induced by the pentavalent compounds.

#### 6 'Resistant' Cases

There is no clinical means of knowing in advance whether any given untreated case of kala-azar will or will not require more than the usual number of injections, so the only sense in which the term 'resistant' can be used in kala-azar is to indicate a case in which previous treatment has failed to effect a cure.



GRAPH 15



# THE SEROLOGICAL CONTROL OF TREATMENT OF KALA-AZAR WITH OBSERVATIONS ON THE SIGNIFICANCE OF HYPOPROTEINÆMIA

BY

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THE eight graphs published in a former paper by the senior writer (1928) showed the effect of a concentrated course of treatment by Bayer 693 B\* upon the serum protein fractions of well-established previously untreated cases of kala-azar, and as a result the view was expressed that these graphs could be used as a serological control of treatment in kala-azar. In order to subject this view to as severe a test as possible, we have applied this protein graph test to a range of varying types of the disease treated in very different ways as will be seen below. We have studied —

- 1 The effect of concentrated courses of 693 B
- 1a The effect of alternate daily injections of 693 B
- 2 The effect of intramuscular injections of 693 B
- 3 The effect of interrupted intravenous courses of 693 B
  - (a) The effect of one injection of 693 B
  - (b) The effect of two injections of 693 B
  - (c) The effect of four injections of 693 B
- 4 The effect of Stiburea
- 5 The effect of sodium antimony tartrate

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\* This substance, p amino phenyl stibinate of diethylamine, is now on the market as Neostibosan



was excellent both clinically and serologically, and it will be obvious that to cure this patient was a considerable achievement. This is an excellent instance of delayed protein response in a very refractory case.

Based on the analogy of other diseases, it is quite conceivable that the avoidance of over-treatment may be of great importance in these refractory cases, for the bodily defensive processes being so greatly weakened by grave degrees of infection may be totally broken down by excessive treatment.

Graph 16 is another excellent instance of a very refractory case.

The case is a very grave one of two years duration and is associated with marked leucopenia. The patient had had approximately 125 injections of many kinds of antimony before admission. On admission he was given a concentrated course of 693 B with heavy dosage. The serum proteins were graphed on the 23rd day, and his total globulin and euglobulin were both found to be extremely high. A further heavy course of 693 B was given when considerable improvement occurred as will be seen in the graph. A third heavy course of 693 B was given on the 52nd day which brought the euglobulin down more sharply. The patient remained very debilitated and was taken home for a period. During this period out of hospital he was taken very ill with a temperature of  $104^{\circ}$  on the 85th day and was readmitted to hospital on the 89th day. His proteins, which were estimated that day, did not indicate that he was any worse, but a further set of observations on the 97th day showed a distinct relapse. No further treatment has been given and although there is considerable improvement the graph shows that the patient even after this immense amount of treatment has not yet entered the second stage, and so the prognosis remains uncertain. His temperature fell to about  $99^{\circ}$  for a week after the last course, being somewhat irregular later.

It seems possible that had we applied the moral pointed in the last graph and withheld further treatment after the first course, the resulting graph might have been the same or even more favourable. We had, however, to consider the feelings of the patient and his relatives, in this instance the patient who was an educated man would probably have gone elsewhere for treatment had we withheld injections.

Graph 17 is another good instance of a resistant case which had had much previous treatment. Her history was that she had had fever for two years and had received a course of 2.3 grms urea stibamine in 15 injections. Eighteen months later a further course of 1.9 gm urea stibamine in 15 injections was given, and two months after the second course she received a third course consisting of 0.6 gm aminostiburea. On admission she was given a concentrated course of 693 B in fairly heavy dosage for a female patient. From the 34th day her serum proteins were graphed. Without any further treatment the progress of the graph was quite satisfactory and she entered the second stage on the 86th day, and was then taken away for a holiday with—in view of the graph—an excellent prognosis. It remains mysterious as to why this patient should be so

above They will be taken up in the above order, each type of graph being discussed individually

Details as to the mode of treatment are noted at the foot of each graph The zero day of the graph is the day on which treatment was commenced, previous days being denoted by the minus sign

All the protein fraction estimations recorded herein have been made by the refractometric method of Robertson indicated in two previous papers on the subject (Lloyd and Paul, 1928)

Every case included in this paper is a definite case of kala-azar in which leishmania has been demonstrated

1 *The standard graph produced by a concentrated course of eight daily injections of No 693 B*

By a concentrated course of 693 B we mean in this paper a course consisting of eight daily intravenous injections, the first dose being 0.2 gm and all subsequent doses being 0.3 gm, i.e., a total of 2.3 grms The graph produced by this concentrated course is of absolutely fixed type which we may conveniently designate the standard graph A very characteristic feature of the standard graphs is their extremely regular form

Graph 1 is an absolutely typical standard graph showing all the special points The initial changes termed the first stage comprise rapid crossing of the albumin and total globulin graphs, double intersection of the pseudoglobulin and euglobulin graphs, the point of second intersection corresponding with the point at which the albumin and total globulin values first stabilize themselves The globulin/albumin ratio has at this point fallen to normal (0.66 to 0.64) Next comes a second stage characterized by a steady ascent of the pseudoglobulin and a steady descent by an equal gradient of the euglobulin, the latter finally falling to the normal figure of approximately 0.16 gm per 100 cc of serum During the second stage the albumin and total globulin values remain absolutely steady The first stage occupies about one month, and the second stage about three months A further feature is that the euglobulin falls gradually during the first stage and more sharply in the second stage

Clinically the response of this patient to treatment was good, the temperature falling to normal by the ninth day and being subsequently as high as 99° occasionally

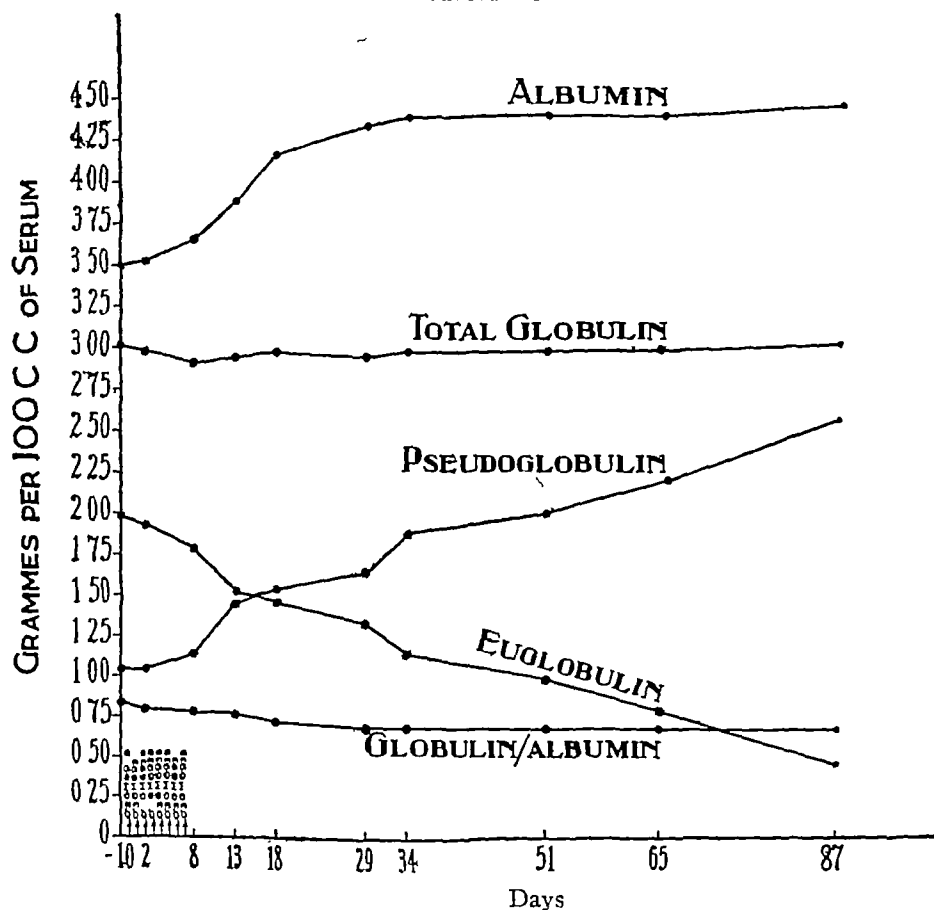
1a *Treatment by eight injections of 693 B on alternate days*—(First injection 0.2 gm, all subsequent injections being 0.3 gm Total dosage = 2.3 grms)

Graph 2 shows a case with high total globulin and euglobulin values before treatment exactly as does Graph 1 It was, therefore, specially chosen for comparison of the two treatments It will be seen that the essential changes are the same, but there are some differences

The sudden fall in the pseudoglobulin in the initial stage of treatment is not seen The fall in the globulin and rise in the albumin are slower, so the intersection takes place later Also the onset of the second stage is much

There is no question here of the crossing of the globulin and albumin curves, but it will be noticed that the globulin which was 3 grms per 100 c c of serum before treatment continued at about the same level, whereas the albumin which is much below the normal increased under treatment. As this case evolved the euglobulin has increased at the expense of the pseudoglobulin, this feature undergoing reversal as the result of treatment.

GRAPH 18

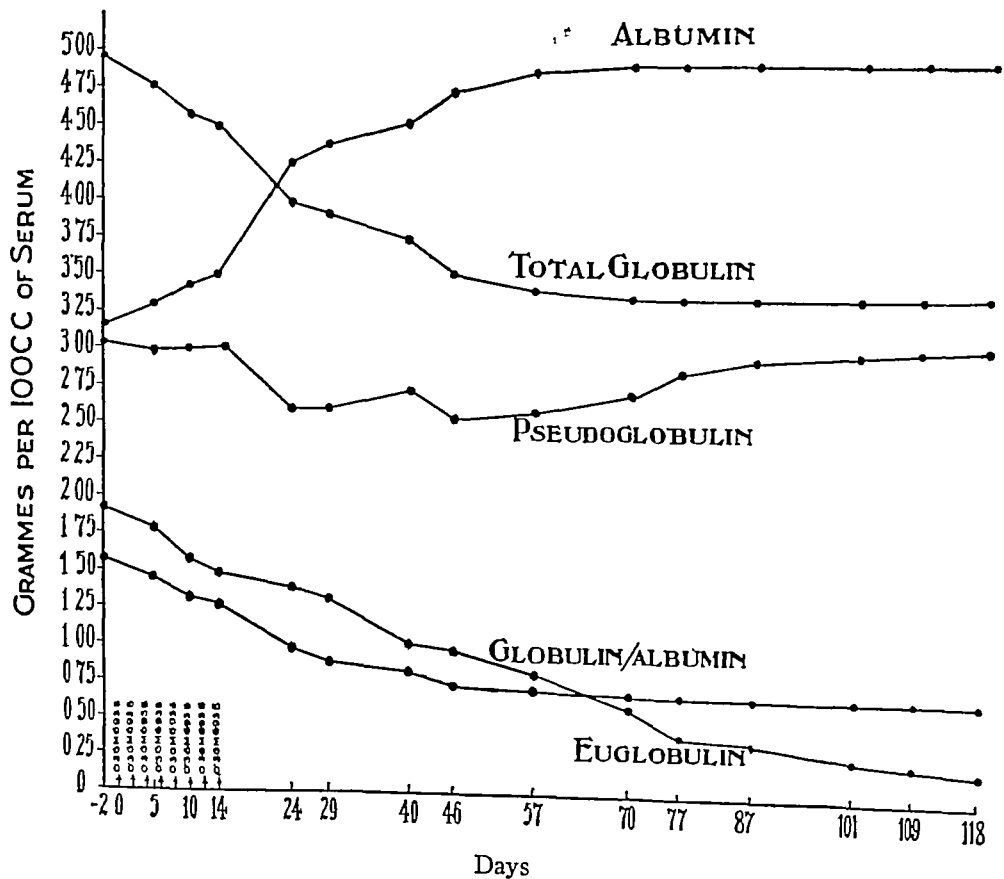


Graph 19 shows another case with only a very weak formol leucogel reaction before treatment. Again we see the association with hypoproteinaemia, the total proteins being the extremely low figure of 5.40 grms per 100 c c of serum. This case had not had previous treatment, and was of about six months duration, i.e., amply sufficient time to generate a strongly positive formol leucogel reaction. The clinical response was distinctly less satisfactory than usual, the temperature not becoming normal until the 20th day and even then being slightly irregular subsequently. The increase to normal of the albumin under treatment will be noticed. The temporary relapse of the pseudoglobulin during treatment is an unusual feature.

The onset of this case was of the malarial type. The leucogel reaction gave a fully positive result in one minute. The temperature became normal on the ninth day. He was discharged on the 32nd day, during his stay in hospital he gained 15 lbs in weight.

2 Treatment by intramuscular injections of 693 B (concentrated course of nine daily injections)

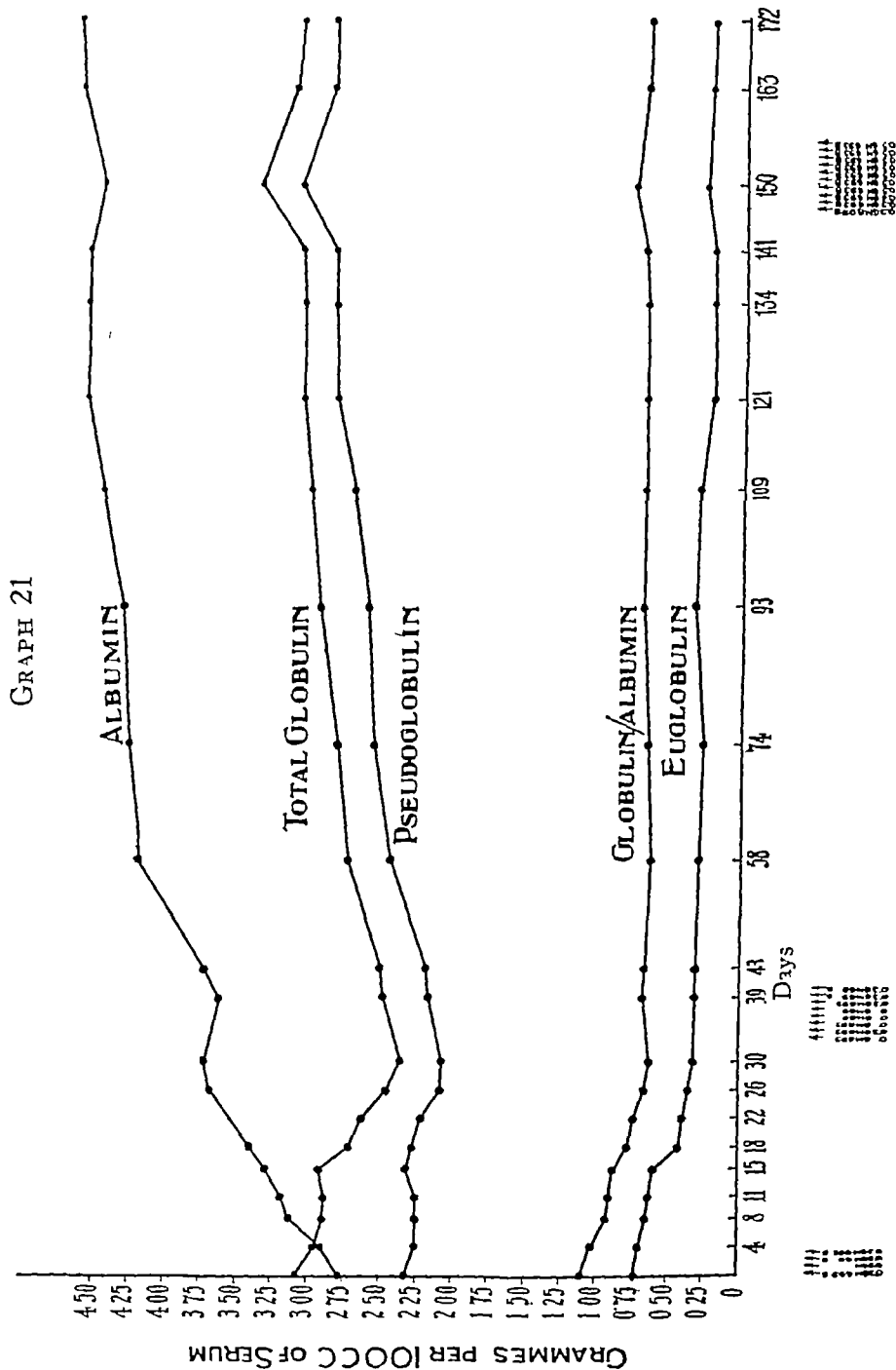
GRAPH 3



It will be seen in Graph 4 that in spite of daily injections there was no protein response for three days, but then the usual changes occurred producing the typical graph. This case did very well serologically. Formalin gave only a clear gel on the 100th day, and by the 137th day the patient was serologically cured with the protein fractions normal and no gel with formalin. It is clear from this graph that intramuscular treatment of this type of case with high total globulin and euglobulin figures is effective in the usual dosage.

The treatment given to this patient varied slightly from the normal course as he developed an urticarial rash half a minute after the first intramuscular injection of 0.2 gm. The second dose was accordingly reduced to 0.1 gm, the third to 0.2 gm, and the fourth to 0.25 gm. After this, four injections of

was considered advisable to give a second full course after which the temperature became normal in six days



A tendency to relapse in the globulin/albumin ratio will be seen commencing on the 29th day This is the point at which the signs of cancrum oris appeared

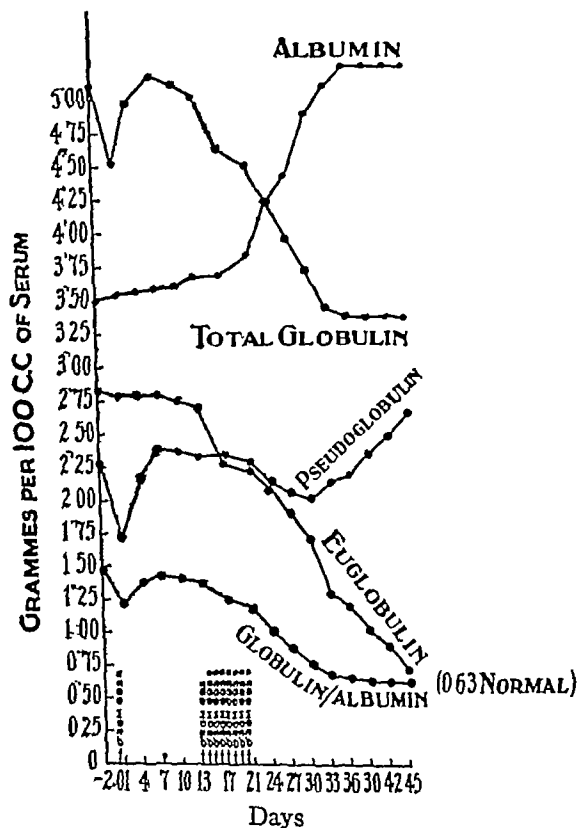
margin, this was reduced to  $3\frac{1}{2}$  inches at the time of his discharge, and when last seen was only just palpable. He gained 6 lbs in weight.

### 3 The effect of interrupted intravenous courses of treatment by 693 B

These were carried out partly to study the effect on the graphs, and partly to form an opinion as to minimum possible dosage.

(a) The effect of one injection of 693 B followed after an interval by the usual concentrated course—Graph 5 shows a typical case with high total globulin

GRAPH 5



and euglobulin values. A single injection of 693 B caused the tremendous drop in the pseudoglobulin so constantly met with in treatment by concentrated courses of 693 B, the euglobulin and albumin being but little changed. It will be seen that after this the proteins quickly relapsed, until seven days after the single injection the globulin was actually higher than before. On the 13th day a full concentrated course of 693 B (as defined above) was given. This produced the usual response, and by the 30th day the patient was well on the way to cure with the upward turn of the pseudoglobulin and the downward progress of the euglobulin.

The history of this case was fever of about one year's duration with an insidious onset. The clinical progress was good, the temperature becoming normal on the last day of the full course.

producing a quite regular protein graph. It almost seems that the wider the departure from normal of the globulin figure, the sharper is its descent under treatment. Having in view these clinical and serological facts it seems to us that these protein changes, so characteristic of the well-developed case, may be of the nature of an immunity response, and that the well-established case having taken some steps towards curing itself, so to speak, responds very rapidly to treatment.

(3) *The form and meaning of the graphs*—We see that the general form of the graphs is not dependent upon the use of any one mode of treatment, those produced by sodium antimony tartrate, stiburea and *alternate daily injections* of 693 B all being substantially similar. The standard graph produced by concentrated courses of 693 B exhibits one additional factor, viz., the sudden fall in the pseudoglobulin in the initial stages of treatment.

So far as we have seen, the sharp dip in the pseudoglobulin occurs only when well-established cases are treated by concentrated courses of 693 B. The reason for this is not clear. It is not shown by all cases so treated, though it is shown by most of them. It tends to be absent in 'resistant' cases. Now all kala-azar remedies are toxic to some extent, and a concentrated course of 693 B in the dosage used is probably the most potent mode of treatment yet devised. Serologically, the fall in the total globulin, of which the pseudoglobulin fall is a part, is more rapid and the second stage is more quickly entered with concentrated courses of 693 B than with any other treatment. In view of these facts we incline to the view that the pseudoglobulin fall is part of the process of cure and is not a toxic phenomenon, even though, when the case eventually reaches serological cure, the pseudoglobulin is higher than before treatment.

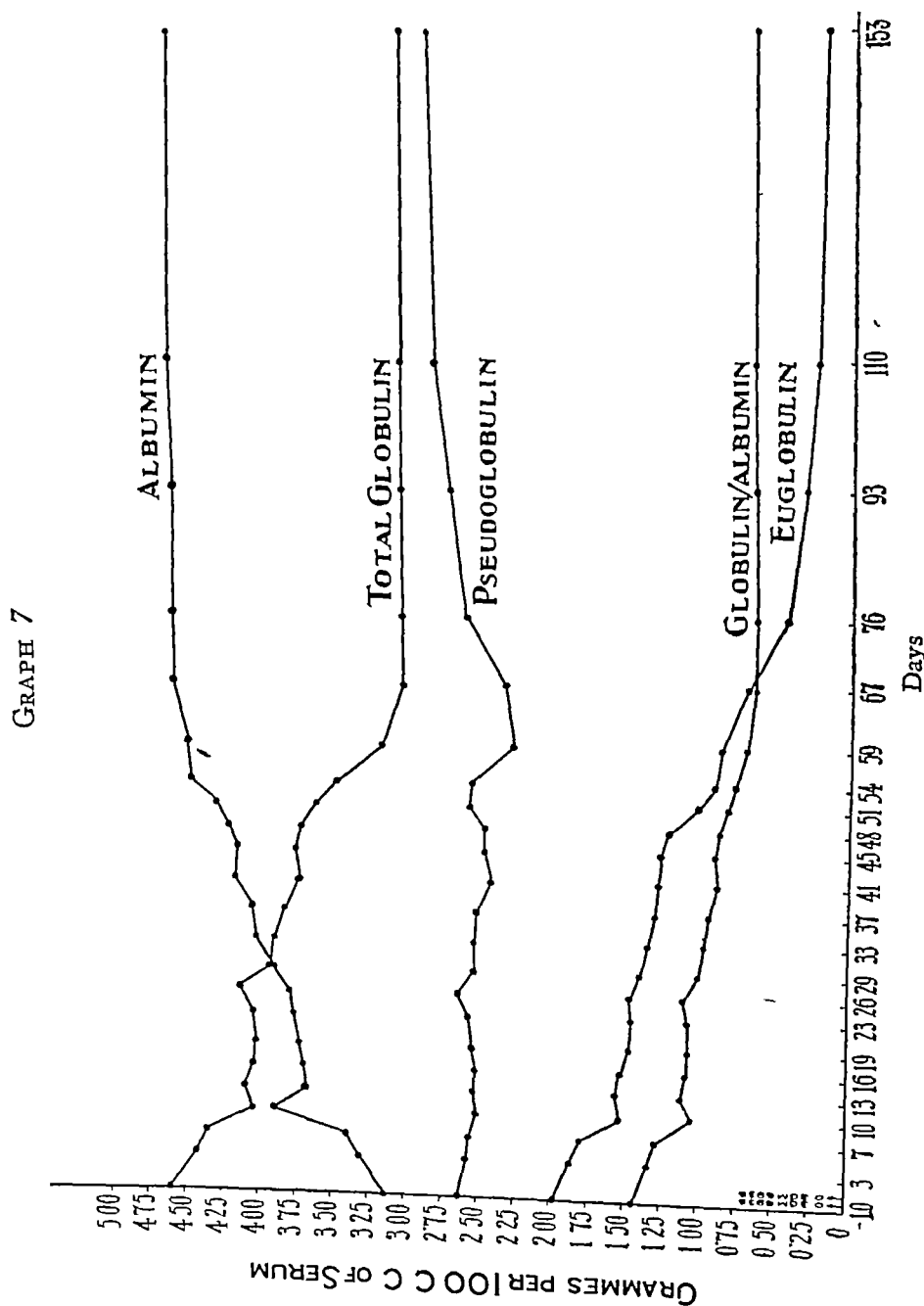
In a well-established case of kala-azar the pseudoglobulin is usually somewhat below the figure for normal serum, and it is curious that the first effect of treatment should be to reduce it still further.

The general form of the graphs, so far from being peculiar to any particular method of antimony treatment, is not special to kala-azar. In a paper by the senior writer now under preparation it will be shown that cases of secondary syphilis treated with organic arsenicals exhibit somewhat similar curves, although they do not show the heavy pseudoglobulin fall in the initial stages of treatment. It may perhaps be anticipated that similar graphs will be met with in other diseases in which the globulin is higher than the albumin.

Two very striking features of the standard graphs are their extraordinary concordance one with another and their remarkable freedom from minor irregularities opposed to their general direction, e.g., a typical case under treatment by a concentrated course of 693 B shows in the second stage a regular increase in the pseudoglobulin to normal without any undulations on the curve. These are important practical points, for were it otherwise, variations from the normal could not be used as a therapeutic guide. The protein graph is probably a purer picture than the clinical observation of the patient in that it is not distorted by

of serum), and the other protein fractions normal in amount and no gel with formalin

The history in this case is of eight months fever with a malarial type of onset. The clinical progress was good, the temperature becoming normal on the



last day of the full course. He gained 7 lbs in weight during his stay in hospital and has subsequently gained an additional 11 lbs.

(b) *The effect of two injections of 693 B*—Graph 7 is an extraordinarily interesting graph. Two injections each of 0.3 gm produced the usual fall in



at present is that once a fairly adequate course of treatment has been given and the globulin/albumin ratio is still falling, no matter how long afterwards it may be, further treatment is not usually required, and it would seem that once a patient is in the second stage with the pseudoglobulin ascending, he will probably require no further treatment. In other words, from the time the second stage is entered the prognosis is good.

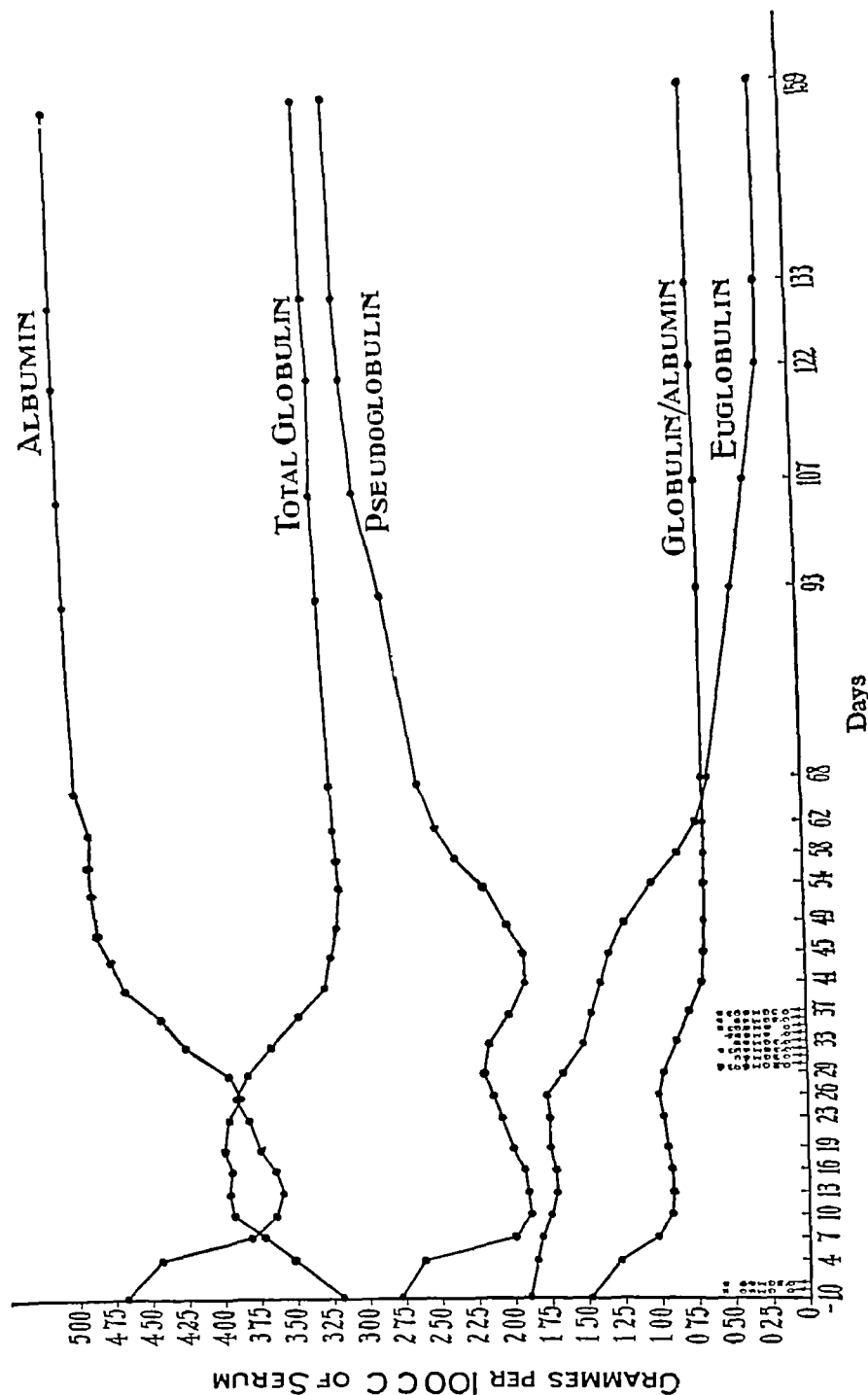
(4) *The relative value of different treatments*—The information provided by the graphs opens up a large question as to the possibility of measurement of the relative efficiency of different drugs and of different plans of treatment. Kala-azar patients cannot be divided into severe or mild, in so far as their response to treatment is concerned, except in the 'resistant' cases, i.e., cases which have failed to respond properly to a previous course of injections in doses usually adequate. Thus, if a kala-azar patient has never had a course of treatment before, there is no means of ascertaining whether he will or will not require more or less than the average number of injections, though, as previously stated, one of the writers has the impression that the cases with a moderate length of history react better than those with a short history. In the circumstances the only plan up to the present time has been to give standard courses to all cases. It may be hoped that the protein graphs will give indications as to whether a *therapia sterilans magna* should be aimed at, or whether it is better to give smaller doses more widely spaced.

Whatever may be the explanation of the phenomena of the first stage of the standard graph, it seems clear that the second stage which is characterized by a change in the solubility of the bulk of the globulin is the final process of cure. Consequently, it might be thought that the speed of onset of the second stage would provide a measure of the therapeutic value of the treatment given, e.g., we see from the graphs that 2.3 grms of 693 B divided into eight alternate daily injections produces in the high globulin type of case about the same speed of onset of the second stage as 2.45 grms of stiburea divided into ten alternate daily injections, i.e., from 50 to 60 days, whereas 2.3 grms of 693 B divided into eight *daily* injections produces the onset of the second stage by about the 23rd day in most cases examined.

Graph 7 shows that where only two injections of 693 B were given the onset of the second stage was delayed to the 59th day.

Comparisons of this kind could only be valuable if carried out over a very large series so arranged that the cases were, as far as possible, of equal severity, a particularly difficult matter to ensure in kala-azar as has been previously stated. It must further be remembered that the time taken to reach serological cure is on the whole remarkably constant, being for nearly every case examined about 120 days, no matter what kind or amount of antimony treatment is employed. The very refractory cases may however take much longer (eight months in one case). With due regard to these limitations, very useful information may probably be obtained in this way as to the relative efficiency of the various methods of treatment.

GRAPH 8



(c) *Treatment by four doses of 693 B*—This patient gave a history of fever of insidious origin for only six weeks, but in view of a fully positive leucogel reaction the case was evidently of much longer standing

Graph 9 shows that four doses of 693 B were amply sufficient to cure this patient as judged by the graph. After a slight hesitation on the 25th day the total globulin curve definitely turned downwards, and the patient entered the second

the disease syndrome This is a hypothesis regarding which further evidence will be required

In India kala-azar is always associated with malaria, and for many years was looked upon as a severe form of this disease, it seems possible that a malarial attack, or an attack of some other disease, such as typhoid, in which the protein content of the serum is low,\* is an essential predisposing factor, and that once the parasite of kala-azar has invaded the system a vicious cycle is established whereby the albumin is maintained at a low level and the euglobulin increases, until the cycle is broken by the antimony treatment

This suggestion regarding the part played by other diseases in the determination of the morbidity of the leishmania infections is not new and has already been discussed at some length by one of the writers (Napier, 1927), but we are now putting forward the additional suggestion that this predisposing condition is in some way connected with the reduction of the albumin content of the serum

The senior writer has found that the characteristic protein graph in malaria exhibits a low total of protein,† the albumin being greatly reduced and the globulin slightly reduced Under quinine treatment both curves rapidly rise to normal and parasites disappear from the peripheral blood If kala-azar supervenes, the globulin curve rises above normal and again the malarial parasites disappear from the peripheral blood It is very seldom indeed that during a kala-azar attack malarial parasites are found in the peripheral blood, but once the antimony treatment has taken effect typical malarial attacks are common, so much so that quinine is often given as a routine measure in order that the patient may not be alarmed by a sudden rise of temperature during his convalescence A good chart of this will be found in a recently published monograph on kala-azar (Napier, 1927, p 180)

It would seem that both parasites exist under conditions associated with a low total protein content of the serum, that the low albumin content is maintained by both parasites, that in the case of kala-azar there is a subsequent increase in the globulin factor due to the reaction of the body in the presence of the parasite, which may possibly be evidence of an immunity response, and that this latter change is unfavourable to the malarial parasite which, therefore, temporarily disappears from the peripheral circulation to reappear in many instances when, with the fall of the globulin content of the serum under treatment, conditions again become more favourable

The senior writer has found that cases of secondary syphilis treated with organic arsenicals exhibit graphs extremely similar to those obtained in kala-azar † He has further found, as previously stated, that in malaria the total serum proteins are very markedly reduced, especially the albumin Taking into consideration the fact that induced malaria not only improves cases of metasyphilis clinically, but also beneficially affects the serological changes, the chief of which

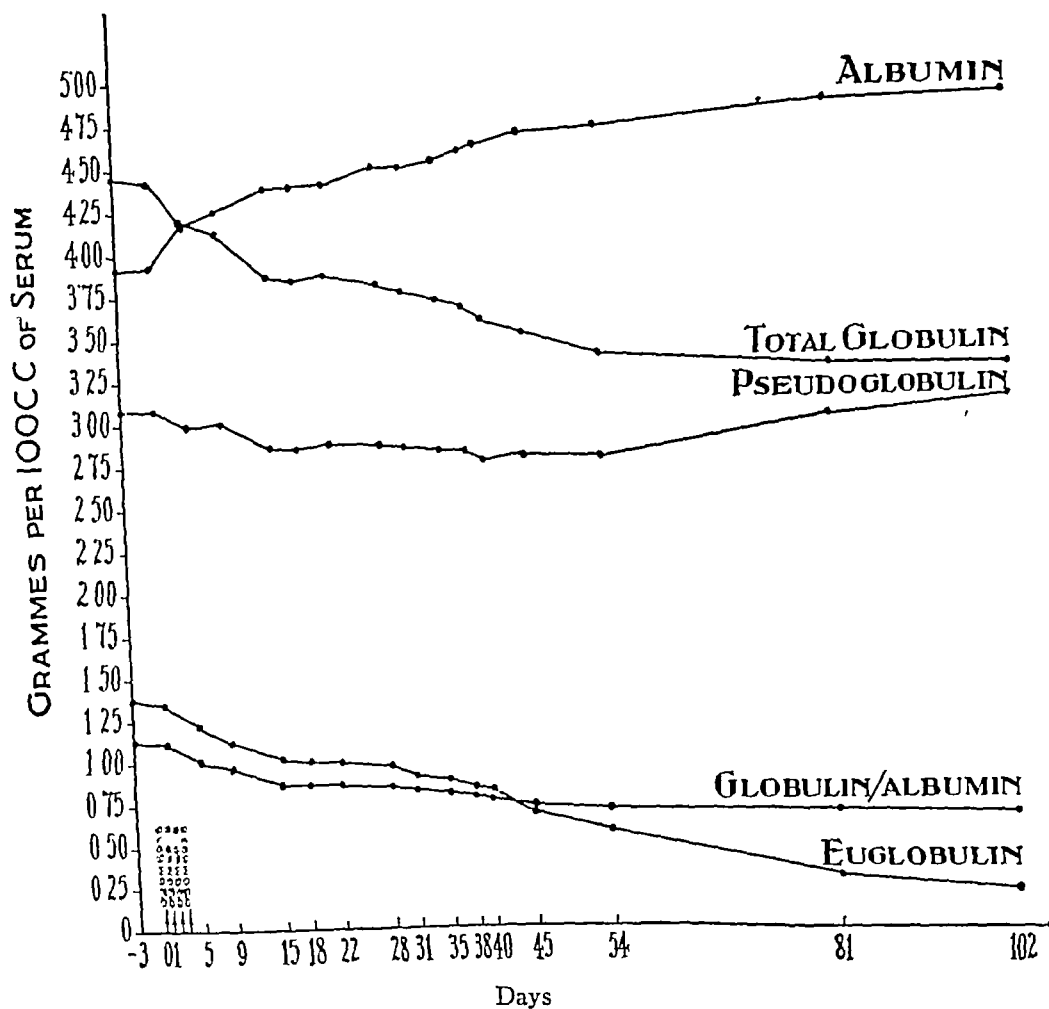
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\* Unpublished observations by the senior writer

† Unpublished observations.

99° This patient had, as already noted, been treated outside with 18 injections of urea stibamine without any improvement, the last injection being only one month before the present course of treatment which, although only a half course, cured him promptly We are unable to explain why this should be

GRAPH 10



These graphs showing the serological effect of fractional courses of 693 B are evidence of the marvellous potency of the pentavalent antimony compounds in kala-azar We are unable to call to mind any other instance at all comparable They certainly far surpass any antisyphilitic drug we possess at present

#### 4 Treatment by Stiburea (urea stibamine)

The first point about Graph 11 is its marked similarity to Graphs 2 and 3 which show the effect of alternate daily injections of 693 B, indicating, as might be expected, that the general form of these graphs is not dependent on the use of any one particular remedy The treatment here consisted of ten alternate

We see from the above table that of the total of 255 cases 18 relapsed. Of these 18 relapsing cases, 13 were associated with a weak or absent formol leucogel reaction. Taking the groups separately, of those treated by 471 the weak leucogel class shows a relapse rate of 4 in 41 or approximately 10 per cent, as against a figure for the strong leucogel class of 3 in 59 or approximately 5 per cent.

Of those treated with stibamine glucoside, the weak leucogel class shows a relapse rate of 5 in 21 or approximately 25 per cent, as against a figure for the strong leucogel class of 2 in 34 or approximately 6 per cent.

Taking the group treated by 693 B, the weak leucogel class shows a relapse rate of 4 in 35 or approximately 11 per cent, as against no relapses in 65 cases with a strong leucogel reaction.

The connection between the tendency to relapse after treatment and a weak formol leucogel reaction before treatment is thus very marked. It must be borne in mind that the weak formol leucogel class includes not only those cases in which the assumed immunization has failed to occur, but also early cases in which it has not had time to develop.

The term 'immunization' as used in this connection is of course only intended to express very generally the possible nature of the changes, for we know of no protein antigen which takes five months to evoke the corresponding antibody, which is the length of time required to produce a fully positive leucogel reaction.

#### SUMMARY OF RESULTS AND CONCLUSIONS

(1) Twenty-one graphs showing the behaviour of the serum protein fractions in varying types of kala-azar cases under different treatments are given. These strongly support the view put forward by the senior writer in a previous publication that these changes may be used as a serological control of treatment in kala-azar. This will lead to enhanced precision in treatment and sharply determines the point of cure.

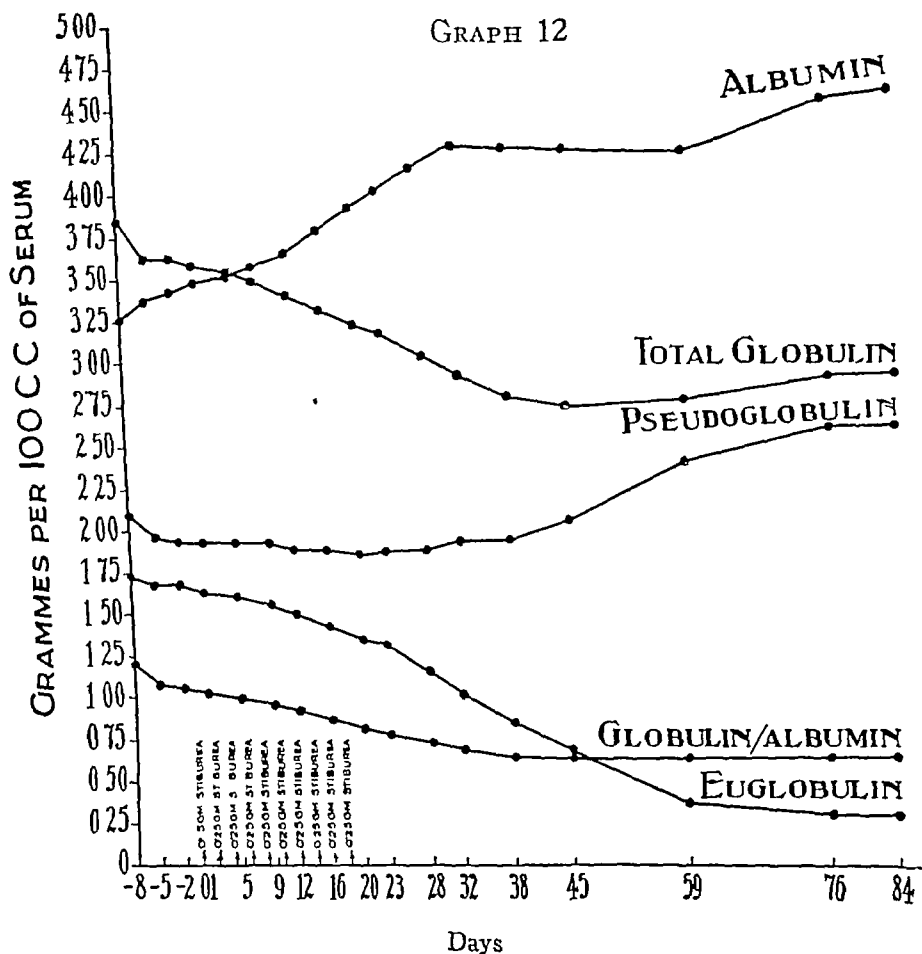
(2) After highly characteristic changes described in the text, the serum proteins resume their normal values approximately 120 days after the commencement of treatment. This point is termed 'serological cure'. At this point the formol leucogel reaction is extinguished.

(3) The protein graph test is much more sensitive than any test previously available, as at any stage of the case it shows whether progress towards cure is occurring or not, whereas clinical appearances do not allow of accurate metrical notation and are subject to extraneous influences, such as mild intercurrent infections, which are not reflected in the protein graphs. So long as the curves are progressing towards normality, we are of opinion that further treatment should not be given. Relapse of the curves indicates that further treatment is required. A very important application of the graph test is the prevention of over-treatment, which may quite possibly prejudice the chances of recovery in grave degrees of infection.

The history was 5 months illness with a malarial onset

### 5 Treatment by Sodium Antimony Tartrate

Graph 13 is of very similar type to Graphs 11 and 12 indicating that the general form of the graph is not limited to the effect produced by the pentavalent compounds of antimony. After receiving 16 injections, that is only half the usual course, the patient insisted on leaving hospital. His clinical progress had not been particularly favourable, as he still had some fever when he left the hospital.



On the other hand, his serological progress was very favourable, the second stage being entered on the 31st day and serological cure being reached on the 96th day, unusually early. His subsequent clinical history—he now appears to be completely cured—suggests that in this instance the serological findings gave the sounder prognostic indication.

Graph 14 shows another case treated with 13 alternate daily injections of sodium antimony tartrate. The first response was quite typical and the patient appeared to be progressing satisfactorily, but on the 21st day both the total

mimical to the malarial parasite. Although in India malaria is always associated with kala-azar, malarial attacks do not occur while the kala-azar serum is in the high globulin condition, they are, however, common when the globulin figure returns to normal under antimony treatment.

(15) In cases of kala-azar treated by concentrated courses of 693 B, the parasites are not usually obtainable by spleen puncture after the 30th day. This approximately corresponds to the time of onset of the second stage, thus supporting our hypothesis that with the rise of the albumin and fall of the globulin the serum no longer offers a favourable medium for the growth of the kala-azar parasite which accordingly dies out.

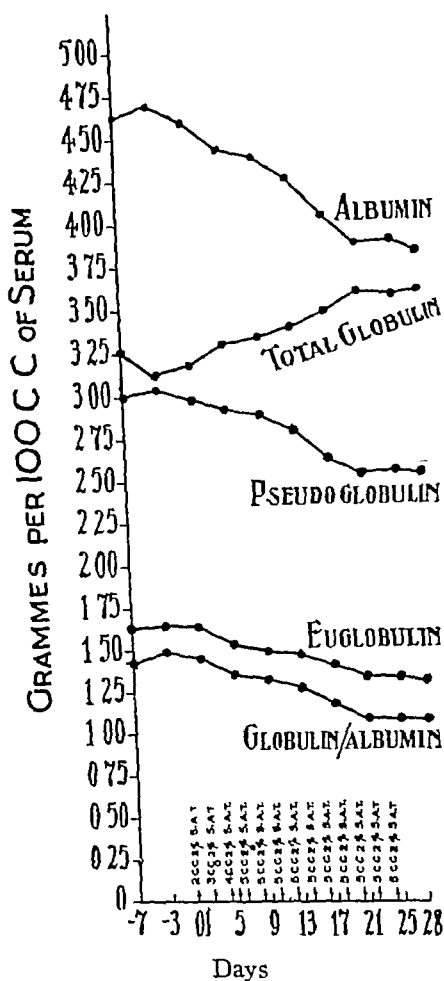
(16) Apart from their applications to the treatment of kala-azar and the help they may possibly give in solving the problem of the transmission of the disease, the graphs are of a certain basic value as indicating the type of response which may be expected when high globulin conditions are treated with the appropriate remedy.

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| <i>Idem</i> (1928)                  | <i>Ibid</i> , Vol. XVI, No. 2, p. 529  |
| NAPIER, L. EVERARD (1927)           | 'Kala-azar, a Handbook for Students and Practitioners' Oxford University Press |
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Graph 15 is a very interesting case The patient was gravely ill on admission and was expected to do badly He had been previously treated with two courses of sodium antimony tartrate and two courses of pentavalent antimony After one concentrated course of 693 B with dosage rather larger than usual he absconded

GRAPH 14



and reappeared on the 63rd day very much better, his weight having increased from 70 to 83 lbs He had no treatment while out of hospital When he returned his serum proteins were again graphed, and his euglobulin being high he was evidently far from cured He was observed for a time, and without any further treatment the typical protein response commenced, i e, the albumin and total globulin curves crossed in the usual manner, the euglobulin began to fall and he entered the second stage about the 105th day, after which the upward movement of the pseudoglobulin began, associated with the corresponding fall in the euglobulin, and he reached serological cure on the 236th day, that is after nearly eight months This is double the time required to produce a cure in the ordinary case The practical value of the graph in this case was that it showed



which are capable of varying degrees of derangement in many pathological conditions was originally described by Aschoff and Landau in 1913 as the 'reticulo-endothelial metabolic apparatus'. Previously, a succession of workers had gradually laid the foundation for this comparatively recent conception.

From very early times histologists have recognized the existence of phagocytic mononuclear cells in the connective tissue of the body. About half a century ago Ranvier described a group of cells which he called 'clasmatocytes' since he considered that they performed their function of carrying nutritive material in the tissues by a process of splitting up and giving off portions of their protoplasm. He believed that these cells were possessed of highly phagocytic properties, and that they were derived from the free cells of the connective tissues, and especially of the omentum.

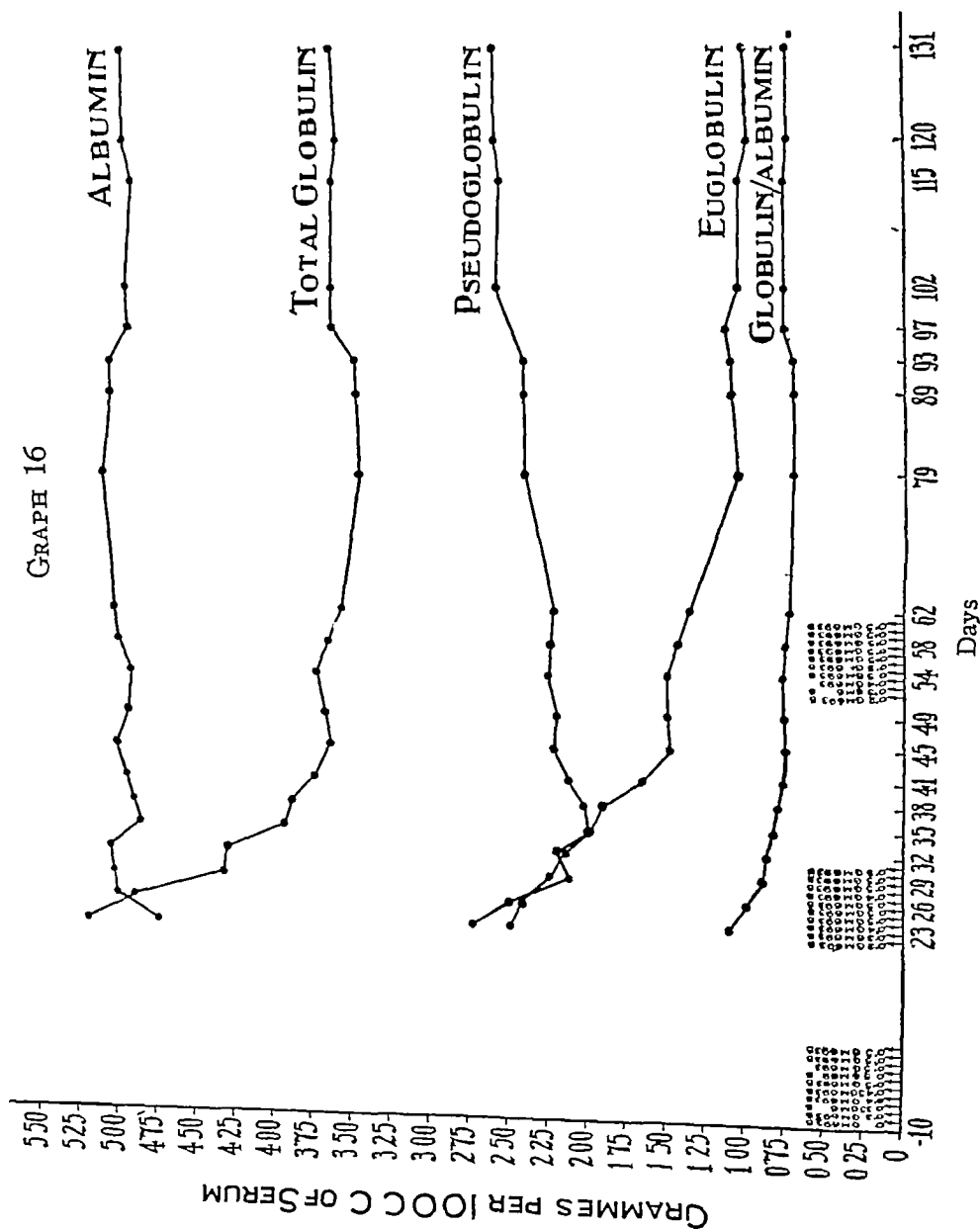
Ranvier's observations were carried a step further by Metchnikoff who described a system of 'macrophages,' and drew a line of distinction between the phagocytic cells of the circulating blood and the relatively fixed and highly phagocytic cells of the tissues. In the former group Metchnikoff included only the large mononuclear cells, and specifically excluded the lymphocytes, while in the latter group he included the large mononuclear cells of the splenic pulp and lymph nodes, certain endothelial cells including Kupffer's cells of the liver, and some of the connective tissue cells.

Other workers quickly added their contributions to these preliminary observations. Marchand showed that the 'clasmatocytes' were derived from mesenchymal elements in connective tissue, especially that in the adventitial sheaths of the blood vessels ('adventitial cells'), and that in inflammatory processes these cells became converted into 'macrophages'. Maximow demonstrated that certain amoeboid mononuclear cells which he called 'polyblasts' were constantly present in inflammatory tissue and considered them to be derived in part from the lymphocytes of the circulating blood, and in part from mobilized local 'resting wandering cells'. Mallory in his studies on typhoid fever found large phagocytic mononuclear cells in the inflamed Peyer's patches, and showed that these cells which he called 'endothelial leucocytes' and similar cells in the mesenteric lymph nodes, liver, and spleen, originated from lymphatic or vascular endothelium.

Ribbert advanced this work still further by introducing a method of vital staining with lithium carmine which definitely mapped out a system of highly phagocytic cells widely distributed throughout the body. He was able to show that the anatomical distribution of these cells was, under normal conditions, constant in a given species of animal. Ribbert also found that the cells specially stained by this vital method were the endothelial and reticulum cells of the spleen, bone marrow, and lymph nodes, the Kupffer's cells of the liver, the reticulum cells of the thymus gland, and certain connective tissue cells.

Some years later Goldman proved that the cells which Ribbert had demonstrated by means of his method of vital staining were identical with the 'clasmatocytes' of Ranvier, the 'macrophages' of Metchnikoff, the 'polyblasts' of Maximow and the 'adventitial cells' of Marchand.

that the patient was slowly being cured, although he had had no treatment for months. In view of the past history further courses of treatment would without the graph certainly have been given, which would have been quite unnecessary and possibly harmful. This we regard as one of the most valuable practical applications of the graph, viz, that so long as the proteins are progressing towards normality the clinician will be wise to avoid further treatment. It is impossible in this case to know with certainty what was happening to the serum proteins up to the 63rd day, but it would appear that this was a case so resistant that the

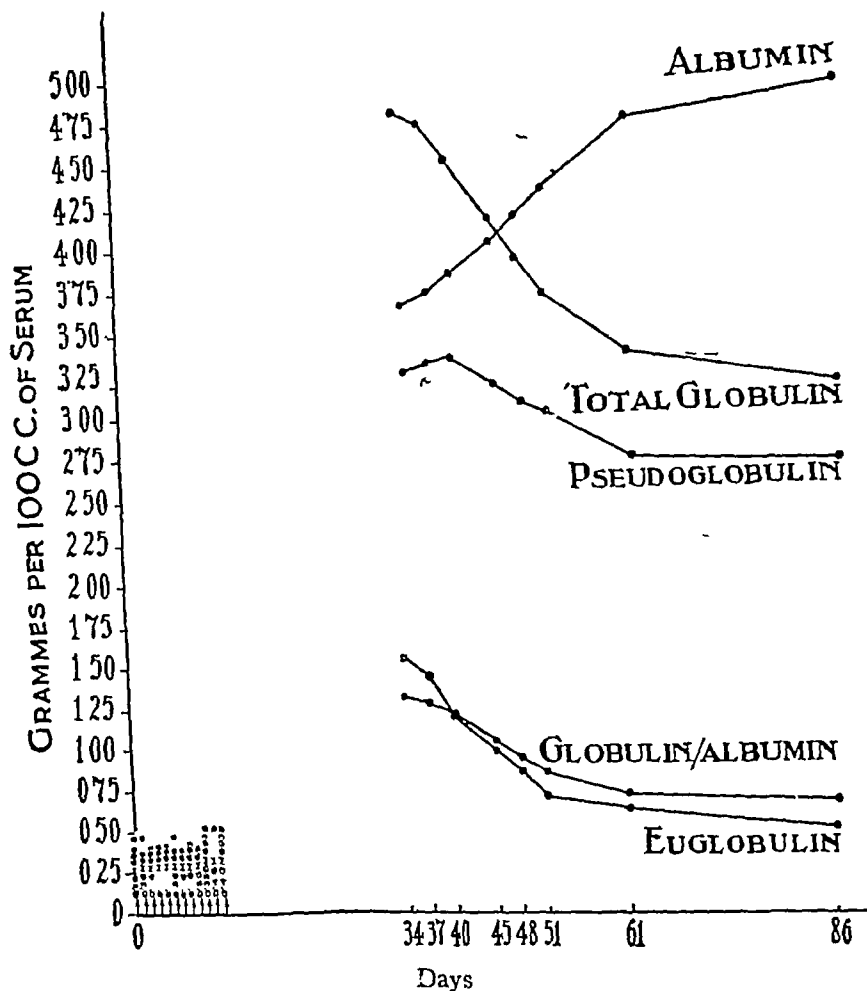


crossing of the albumin and total globulin graphs was delayed to the 78th day, and the patient did not enter the second stage till the 105th day. The final result

quickly cured by 693 B after three previous courses of pentavalent antimony given outside had failed

7 *Atypical Cases*

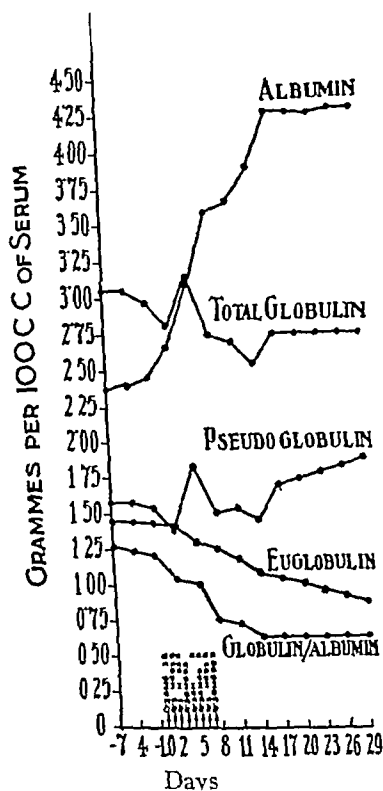
GRAPH 17



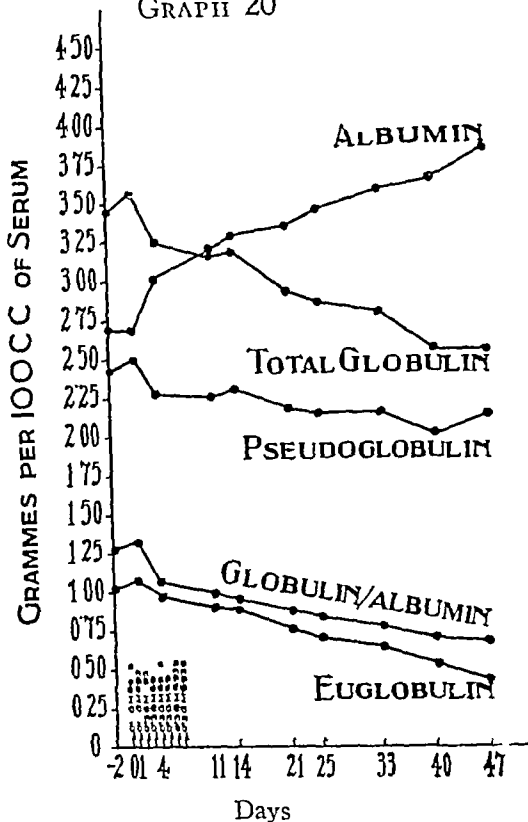
Graph 18 shows a case of kala-azar with the albumin higher than the globulin, associated in this instance with a very high euglobulin and a very low pseudoglobulin. We notice here the low total protein figure of 6.5 grms per 100 cc of serum. This patient also showed an incomplete formol leucogel reaction. A gel with some degree of opacity formed in ten minutes which was unchanged up to 24 hours. The duration was about three months which is too early for the development of a fully positive formalin reaction. No previous treatment had been given. This case gave a fairly satisfactory clinical response, the temperature becoming normal on the 6th day, and the spleen diminished, but he gained no weight and the leucocyte count was only 6,800 per c mm at the time of discharge.

Graph 20 shows a case of short duration of about three months fever with the 'enteric' type of onset, with absolutely no gel with formalin and a completely negative antimony test. Once more we find the low total protein value 6.14 grms per 100 cc of serum.

GRAPH 19



GRAPH 20



This was an acute case with high fever and treatment had to be given at once. The patient was very ill but reacted favourably clinically, the temperature falling to normal on the 8th day. The case was presumably nearing the onset of the second stage after the 47th day when the patient unfortunately ceased to attend.

It will be observed that the onset of the second stage was delayed until after the 47th day, although this patient received a concentrated course of 693 B, which ordinarily produces a second stage after about 23 days. This doubling of the length of the first stage is very distinct evidence of a weak response to treatment.

Graph 21 shows another case with very low total proteins, 5.84 grms per 100 cc of serum. In this case there was the enteric type of onset and the disease was of one year's standing, yet there was a very weak formal leucogel reaction with only partial opacity after one hour. The patient was first given four doses of 693 B in order to see if a half course would produce a cure (compare Graphs 9 and 10). The patient did not do well. After the first course the temperature did not settle to normal and some indication of cancrum oris developed, and it

The large increase in the total protein produced by treatment will be noticed. He was discharged from hospital but two months later he was readmitted. He had not lost weight, but was having occasional fever and his spleen was again enlarging. Parasites were still present as demonstrated by spleen puncture. A reference to the graph shows that after the second course of treatment the euglobulin relapsed somewhat, eventually falling again almost to normal by the 121st day. By the 141st day there was again evidence of a rising euglobulin, and the next observation taken on the 150th day, after a third course of treatment had been commenced, showed a very definite relapse in all the protein fractions. The last course of treatment has apparently brought about cure. It would appear from this case that the association of a long history with low total proteins and a weak or absent formol leucogel reaction is not of good omen.

### DISCUSSION

(1) *The effect of treatment on the parasites*—When sodium antimony tartrate was used in the treatment of kala-azar the full course lasted from two to three months, and it was considered essential that at the end of this course of treatment the spleen puncture should not show the presence of parasites, if it did, the patient almost always relapsed. When the pentavalent antimony compounds were introduced, the average period of treatment was reduced to about a month, and it was found that at the end of the full course parasites were still sometimes present in the spleen-puncture material but that their presence was not an indication that a relapse would necessarily occur. More recently we have found that at the end of the eight-day course of treatment by 693 B—a course which produces a very high percentage cure rate—parasites are almost always present, but that the longer the interval between the commencement of treatment and the spleen puncture the smaller are the chances of parasites being found. A few figures collected from recent cases show that —

Of NNN cultures taken from spleen or liver puncture material from the 18th to 24th day, 11 out of 29 (or 38 per cent) were positive.

Of NNN cultures taken from spleen or liver puncture material from the 24th to 30th day, 3 out of 13 (23 per cent) were positive.

After the 30th day they are usually absent. Referring to the protein graphs, it will be seen that it is usually about the fourth week that the second stage begins, i.e., the albumin has then reached its maximum. One cannot say which is cause and which effect, but a possible explanation is that when the protein graph reaches this stage the parasites are unable to exist as a general systemic infection.

(2) *A possible immunity response*—One of the writers (L. E. N.) has the impression gained from clinical study that cases of kala-azar of moderate duration tend to react to treatment better than those with a short history, and that in the absence of acute symptoms there need be no hurry to treat the average case. Waiting may even improve the prognosis. The average well-established case is one with high total globulin and euglobulin values and a strongly positive formol leucogel reaction. This type of case responds rapidly to treatment.

extraneous factors unconnected with the prognosis. In the average well-established case under treatment there is little or no clinical change after about 40 days, the subsequent phase of steady progress being obscured. The graphs, on the other hand, show serum protein changes occurring up to about 120 days, the average period in which the serum protein fractions become quantitatively normal. At this point the serum is presumably normal serum, the formol-leucogel reaction being entirely extinguished, and we may speak of this point as serological cure. The process of serological cure being much more protracted than clinical cure, it would *a priori* be expected that a test based on it would be much more sensitive than clinical observation. The serological test can see, as Harrison so aptly said of the Wassermann reaction, further into the patient than the unaided eye of the clinician.

The statement that any particular serological change may be utilized to express the progress of any case of disease under treatment and to indicate the point of cure, is, in the nature of things, not susceptible of direct proof. All that can be done is to show a constant association between the protein changes sought to be made the basis of the test and the clinical progress of the patient in a large series of cases. We have had very favourable opportunities of observing a large number of cases of kala-azar both clinically and serologically, and we are convinced that this association exists. We have repeatedly been struck by the way in which variations from the standard graph have been in agreement with deviations from the normal clinical progress of patients. There have been occasions too when from clinical examination a doubt existed as to whether any unfavourable symptom was supervening. Examination of the graph proved that the serum response was continuing normally, and it was shown to be a true index by the subsequent satisfactory clinical progress of the case. The point of serological cure is of course equally well, and much more simply, shown by the extinction of the formol-leucogel reaction, but there is no means of using this as an index of progress under treatment. It cannot for example foretell clinical relapse as the protein graph does. Moreover, in a minority of cases the formol-leucogel reaction is negative before treatment. It is the *direction* of the curves which is the valuable indication to the clinician.

The steady progress of the curves to normality is a solid fact which is of the greatest value to the clinician who is perhaps handling a case which has had no treatment for months, and which shows little change from day to day, and the question arises as to whether further treatment is required or not.

The chief points of clinical value brought out by the graphs are noted in the description of each graph. It is in very refractory cases that these curves probably reach their highest value, giving the clinician a clear indication not obtainable by other means as to whether to give further treatment or whether to withhold it. One of the most important applications of the graphs will clearly be to prevent over-treatment. It is particularly in refractory cases that the clinician needs this aid, as clinical appearances do not sufficiently exactly indicate whether the patient is progressing favourably or not. In treating the average case our experience

(5) *Practical applications of the graphs*—A test of this kind is necessarily limited to central laboratories. The ideal would no doubt be to check the clinical progress by weekly protein estimations. It is not essential to make so many examinations as this. It will be necessary to make the initial observations at fairly short intervals to show the X curve and to catch the upward turn of the pseudoglobulin. After that, perhaps examination every fortnight will prove sufficient. In very bad cases not much change need be expected in the graph at first, and frequent protein estimations will not be called for. If the globulin/albumin ratio is above normal, it indicates that the case is still in the first stage. It must not be deduced from this that more treatment should immediately be given, as in very refractory cases the usual protein response may be considerably delayed. In these cases the general drift of the curves is of great importance.

So far as outstation work is concerned, the graphs will probably reach their greatest usefulness by assisting a kala-azar research worker in touch with a central laboratory to estimate accurately the potentialities of particular drugs and particular plans of treatment, and to enable him more closely to standardize courses of treatment for general adoption in rural areas.

In some cases it may be possible to send the patient down to a central laboratory for a limited number of observations, and if only one protein fraction estimation be possible, it should be made on the 30th day, for then with a concentrated course of 693 B the patient should be well into the second stage, and if he is not, the clinician will know that the patient is not doing well.

The refractory cases, after perhaps many abortive attempts at cure, naturally are referred in the last resort to the kala-azar specialist who, assisted by the graphs, will be able to handle them with enhanced precision.

(6) *The significance of low total serum protein values*—We have seen above that there is a minority of cases in which the total proteins are very low. These tend to be associated with a weak or absent formol leucogel reaction. They also show in one way or another a definitely subnormal response to courses of treatment ordinarily adequate. We have accordingly to consider whether these low total protein values are particularly connected with certain types of case, or whether, in view of the fact that the formol leucogel reaction takes four to five months to develop, they represent the early stage (pre-gel phase) of all cases. If the latter is true, we have further to consider whether the reduction in total proteins is due to infection with leishmania or whether it precedes infection with leishmania, i.e., is due to other diseases.

We have unfortunately not yet obtained a protein graph in a case during the first month of the disease, but the earliest graph, taken in the third month, shows the characteristic changes in the globulin/albumin ratio. The euglobulin was increased at this stage, but had not reached the usual level expected in a well-developed case of kala-azar. It is possible that the albumin content of the serum is low from the beginning of the infection, that is to say that a low albumin content is essential before the parasite can invade the system and cause

is high globulin, we may conclude that an antagonism exists between the low albumin type of serum in malaria and the high globulin type of cerebro-spinal fluid in metasyphilis

If our suggestion that the high globulin type of kala-azar represents some kind of immunity response is correct, and this is responsible for the rapid reaction to treatment, and if the low total protein type represents the opposite condition, then the low total protein type should be associated with a weak response to treatment, or to put the same point in another way, cases with a weak or absent leucogel reaction before treatment should show in general a worse response

Examination of the records of one of the writers (L E N) affords distinct evidence in support of this conception

Three representative groups of cases were examined. All the cases in each group had received a full course of treatment. These groups comprised 100, 55 and 100 cases respectively, i.e., 255 cases in all. They were entirely unselected, save that all previously treated cases were excluded from consideration, as not being a fair sample. For various reasons the number of cases in each group which relapsed was found to be the most convenient index of subnormal response to treatment. The details will be found in the subjoined table —

*Table showing the association between the number of relapsing cases and the strength of the formol leucogel (aldehyde) reaction prior to treatment, as compared with the normal relapse rate*

Group	Course of treatment	Number of cases and number of relapses in each group	Formol leucogel complete or nearly so	Formol leucogel weak or absent
I (100 cases)	471 (Stibosan) (normal relapse rate) 10* per cent	Number of cases	59	41
		Number of relapsing cases	3	4
II (55 cases)	Stibamine glucoside (normal relapse rate) 13 per cent	Number of cases	34	21
		Number of relapsing cases	2	5
III (100 cases)	693 B (Neostibosan) (normal relapse rate) 4 per cent	Number of cases	65	35
		Number of relapsing cases	0	4

\* Of those cases treated by 471, the particular 100 cases examined showed only 7 relapses, though the normal relapse rate calculated from a much larger series is 10 per cent



(4) Several instances are given of the cure of kala-azar cases by fractional courses of 693 B employed under the guidance of the graphs

(5) No matter what the kind or amount of antimony treatment given may be, provided it is sufficient to produce a cure, the time required for serological cure is remarkably constant, being approximately 120 days in nearly every previously untreated case examined

(6) The onset of the second stage is much more rapid when concentrated courses of 693 B are employed than under any other mode of treatment so far tested

(7) The onset of the second stage indicates that the case has entered upon the final stage of cure, and, consequently, that the prognosis is good

(8) We are of opinion that the speed of onset of the second stage may be regarded as an index of the potency of the treatment employed, and that, therefore, the relative efficiency of different drugs and of different plans of treatment may be estimated in this way, though, as stated above, in (5) the body appears to possess a power of compensating by increased speed in the second stage for delay in the first, so as to bring about serological cure in about four months in almost all cases

(9) The sudden fall in the pseudoglobulin in the initial stages of treatment appears to be limited to cases treated by 693 B, which in the doses used we believe to be the most powerful remedy now available. The pseudoglobulin in the average well-established case of kala-azar is usually somewhat below normal and it is curious that the first effect of treatment should be to depress it still further

(10) The general form of the graphs produced by all modes of antimony treatment so far tested in kala-azar is substantially the same, it is not limited to kala-azar since cases of secondary syphilis treated by organic arsenicals show the same type of graph

(11) Evidence has been adduced that an association exists between low total serum proteins, a weak or absent leucogel reaction and a subnormal response to treatment

(12) Reasons are given for our view that the high total globulin and euglobulin values and strong leucogel reaction met with in the typical kala-azar case may represent a form of immunity response

(13) We suggest accordingly that possibly hypoproteinaemia is an essential predisposing condition in the absence of which leishmania cannot develop into a systemic infection, and further that this condition of low total serum proteins may possibly be caused or assisted by previous attacks of malaria in which disease the total proteins, especially the albumin, are very low. Typhoid fever, in which the serum proteins are also low, may act in a similar way

(14) Our conception is that hypoproteinaemia, the most characteristic feature of which is marked reduction in the albumin, produces conditions in which the malaria parasite will exist and reproduce, but that if kala-azar supervenes, the subsequent reaction of the body produces the high globulin condition which is

# STUDIES ON THE RETICULO-ENDOTHELIAL SYSTEM WITH SPECIAL REFERENCE TO MALARIA

## Part I

### INTRODUCTORY

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#### I INTRODUCTION

WITHIN recent years the reticulo-endothelial system (R E system) has attracted much attention in many fields of medicine and surgery, but, so far as can be ascertained from the available literature, the relationship of this system to tropical diseases has received remarkably little attention. Hu and Cash (1927) have indicated its importance in kala-azar, and the possibility of its involvement in malaria has been only tentatively suggested by a very few authorities. It is thought that a study of the relationship of this system to other tropical diseases may throw light on many of the obscure problems in tropical medicine.

It has been considered advisable to review the work of the earlier authors whose observations have laid the foundation for the present conception of the reticulo-endothelial system, and to give an account of the anatomical distribution of the cells comprising this system, in the light of the most recent researches.

In the present paper, mention will only be made of the various physiological functions which have been attributed to the R E system. It is hoped in a later series of papers to study the relationship of these functions to malaria, and, as each of these is taken up in this connection, the relevant literature will be fully discussed.

#### II HISTORICAL

The system of widely scattered cells, which has come to be recognized as an organ with a definite anatomical distribution, and certain physiological functions